SOMATIC VARIATION OF CHROMOSOME NUMBERS IN HYBRID WHEATS[1]

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Received June 2, 1938

DURING an examination of meiosis in 336 plants of 50 *vulgare*-like lines, derived from interspecific crosses involving four varieties of *Triticum vulgare* and *T. durum* var. Iumillo, pollen mother-cells with aberrant chromosome numbers were found in plants with 39, 40, 41 and 42 chromosomes.

MATERIAL AND METHODS

The 50 lines used in this study were obtained from Dr. R. F. PETERSON, Dominion Rust Research Laboratory, Winnipeg, Manitoba. The parental varieties were: *T. vulgare* (2n = 42) var's. Marquis, Marquillo (derived from a Marquis × Iumillo cross), Hope (derived from a Marquis × Yaroslav Emmer cross), and R.L. 729 (derived from a Pentad *durum* × Marquis cross); *T. durum* var. Iumillo (2n = 28).

One anther, only, was used in the preparation of each slide, and the observations were made *before* and after the smear slides were made permanent.

OBSERVATIONS

From the results summarized in table 1, it is seen that aberrant pollen mother-cells were found in one or more plants of all crosses studied, and in all generations recorded with the exception of F_7 Iumillo × R.L. 729.

TABLE 1

The distribution of the aberrant pollen mother-cells.

MATERIAL	NUMBER OF FAMILIES WITH ABERRANT PLANTS/TOTAL FAMILIES STUDIED	NUMBER OF PLANTS WITH ABERRANT PMC/TOTAL PLANTS STUDIED
Marquis × Iumillo F_7	1/ 9	1/ 50
Iumillo × Hope F_7	5/12	8/ 82
Iumillo × Hope F_8	1/ 5	2/ 28
Iumillo × R.L. 729 F_7	0/ 4	0/ 28
Iumillo × R.L. 729 F_8	4/ 7	4/ 46
Marquillo × Iumillo F_8	1/13	1/102
Totals	12/50	16/336

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The frequencies of plants with aberrant pollen mother-cells in the various crosses were: Marquillo × Iumillo, 0.98 percent; Marquis × Iumillo, 2.00 percent; Iumillo × R.L. 729, 5.45 percent; and Iumillo × Hope, 9.09 percent (*cf.* table 1). Aberrant pollen mother-cells were found in six of the 140 plants with 42 chromosomes (4.2%), seven of the 98 plants with 41 chromosomes (7.1%), two of the 50 plants with 40 chromosomes (4.0%), and one of the 27 plants with 39 chromosomes (3.7%).

Seventy-six aberrant pollen mother-cells were observed among the 505 pollen mother-cells examined in 16 plants distributed among 12 families. The number of aberrant pollen mother-cells recorded in any one plant varied from one to 20. Their chromosome complements varied from 15 to 61, and the number of bivalent chromosomes varied from three to 29. The data are given in table 2. Details of the chromosome number and arrangement in aberrant pollen mother-cells of two plants are given in tables 3 and 4. Typical aberrant pollen mother-cells are illustrated and for comparison, four "normal" (2n) pollen mother-cells from plants with 39, 40, 41 and 42 chromosomes are also included (Plate I, figures 1–11).

DISCUSSION

HOLLINGSHEAD (1932) reported the rare occurrence of one or a small group of pollen mother-cells with less than the normal number of chromosomes in Marquillo and in a hybrid of *T. vulgare* var. Garnet with an unknown variety.

KATTERMAN (1933) described the occurrence of such hypoploid pollen mother-cells in wheat-rye hybrids. His paper also included a review of the literature on cytomixis, to which phenomenon he ascribed the abnormali-

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DESCRIPTION OF PLATE I

Photomicrographs of pollen mother-cells in *vulgare*-like derivatives of pentaploid wheat hybrids. Aceto-carmine preparations. ×540.

FIGURE 1. Normal cell in a 39-chromosome plant. Nineteen bivalents and one univalent.

FIGURE 2. Aberrant cell in the same plant. Six bivalents and eight univalents.

FIGURE 3. Normal cell in a 40-chromosome plant. Nineteen bivalents and two univalents.

FIGURE 4. Aberrant cell in a 40-chromosome plant. Ten bivalents and ten univalents. Not flattened.

FIGURE 5. Normal cell in a 41-chromosome plant. Twenty bivalents and one univalent.

FIGURE 6. Aberrant cell in a 41-chromosome plant. Six bivalents and eleven univalents. Not flattened.

FIGURE 7. Normal cell in a 42-chromosome plant. Twenty-one bivalents.

FIGURES 8–11. Aberrant cells in 42-chromosome plants.

FIGURE 8. Three bivalents and nine univalents.

FIGURE 9. Five bivalents and ten univalents.

FIGURES 10 and 11. Contiguous cells with a total of 84 chromosomes.

FIGURE 10. Twenty-nine bivalents and three univalents.

FIGURE 11. Ten bivalents and three univalents.

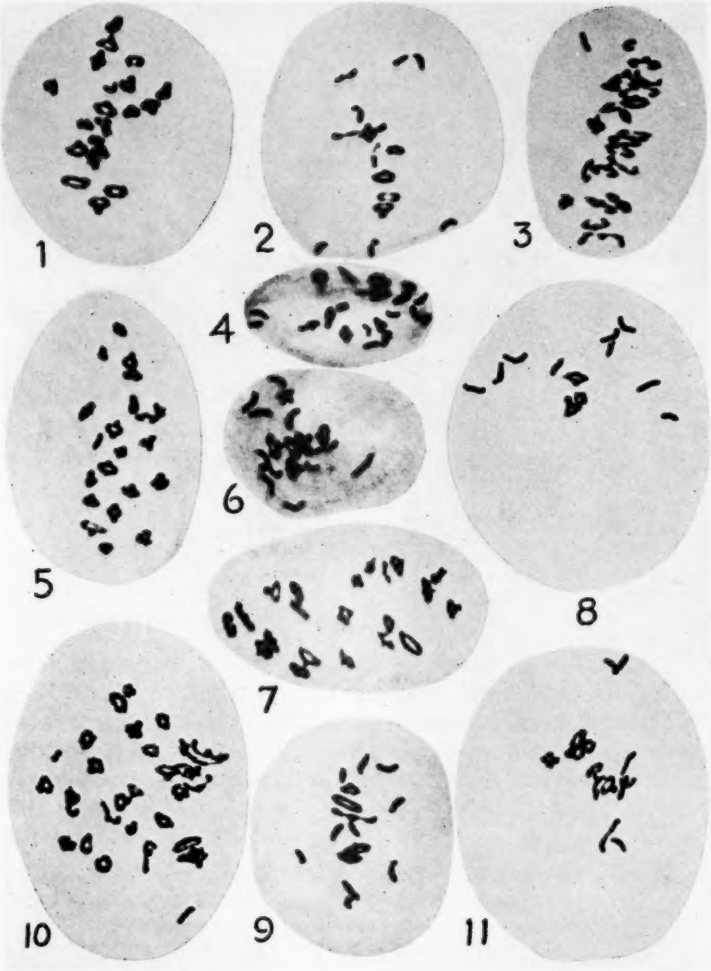
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TABLE 2

The occurrence, and range in chromosome number, of the aberrant pollen mother-cells.

MATERIAL	ACCESSION NUMBER	CHROMOSOME NUMBER AND ARRANGEMENT CHARACTERISTIC OF PLANT*	NUMBER OF ABERRANT/ NORMAL PMC	CHROMOSOME NUMBERS AND ARRANGEMENT IN ABERRANT PMC
Marquis×Iumillo F_7	36–225.8	42 (21_{II})	3/21	20 (5_{II})
Iumillo×Hope F_7	36–233.1	41 ($20_{II}+1_I$)	15/27	20–30 (6–10_{II})
	.7	40 ($19_{II}+2_I$)	2/20	32 (11_{III})
	−235.9	42 (21_{II})	1/20	23 (6_{II})
	−236.2	40 ($19_{II}+2_I$)	1/14	25 (6_{II})
	−241.8	41 ($20_{II}+1_I$)	2/32	22, 23 (5_{II}, 7_{II})
	−242.6	41 ($20_{II}+1_I$)	3/50	16–28 (5–9_{II})
	.8	39 ($19_{II}+1_I$)	2/26	20 (6_{II})
	.9	41 ($20_{II}+1_I$)	20/49	20–30 (4–10_{II})
Iumillo×Hope F_8	36–246.1	41 ($20_{II}+1_I$)	1/26	29 (10_{II})
	.3	41 ($20_{II}+1_I$)	3/20	26 (6_{II})
Iumillo×R.L. 729 F_8	36–249.9	42 (21_{II})	1/30	34 (6_{II})
	−252.3	42 (21_{II})	1/20	25 (9_{II})
	−255.5	41 ($20_{II}+1_I$)	14/31	30 (11_{III})
	−256.7	42 (21_{II})	5/20	15, 27 (3_{II}, 9_{II})
Marquillo×Iumillo F_8	36–271.11	42 (21_{II})	2/23	23, 61 (10_{II}, 29_{II})

* II signifies bivalent, I signifies univalent.

ties found in his material. KATTERMAN found pollen mother-cells with the following irregularities: in the first metaphase division, an excess of chromatin in addition to the normal set of chromosomes; in early diakinesis, secondary nuclei in addition to the main nucleus; and in zygotene and pachytene, cytomixis, that is, the passage of nuclear material from one pollen mother-cell to another.

TABLE 3

Chromosome number and arrangement in aberrant pollen mother-cells of plant 36–233.1 ($2n=41$).

CHROMOSOME NUMBER	ARRANGEMENT	FREQUENCY
20	$6_{II}+8_I$	1
21	$6_{II}+9_I$	1
21	$7_{II}+7_I$	3
22	$6_{II}+10_I$	1
23	$6_{II}+11_I$	5
23	$7_{II}+9_I$	1
25	$7_{II}+11_I$	2
30	$10_{II}+10_I$	1

The writer (1936) recorded the occurrence of two "haploid" pollen mother-cells in a 42-chromosome derivative of the intra-*vulgare* wheat cross H-44 × Reward. Perhaps of some significance is the origin, in similar material, of a 35-chromosome (14 bivalents and 7 univalents) plant from a selfed 42-chromosome plant.

YASUI (1937) found aberrant pollen mother-cells in an artificially produced Papaver hybrid. She observed normal, giant and small pollen mother-cells and attributed the phenomena to hybridity or to aneuploidy. Some of the small pollen mother-cells aborted, and she suggested that this was due to the loss of genes which govern the meiotic process.

TABLE 4

Chromosome number and arrangement in aberrant pollen mother-cells of plant 36-242.9 (2n=41).

CHROMOSOME NUMBER	ARRANGEMENT	FREQUENCY
20	$5_{II}+10_{I}$	1
20	$6_{II}+8_{I}$	2
22	$4_{II}+14_{I}$	1
26	$1_{III}+6_{II}+11_{I}$	1
27	$9_{II}+9_{I}$	5
28	$7_{II}+14_{I}$	3
28	$8_{II}+12_{I}$	3
28	$9_{II}+10_{I}$	1
29	$7_{II}+15_{I}$	1
29	$9_{II}+11_{I}$	1
30	$10_{II}+10_{I}$	1

In the present material no fragments or extra chromatin were found in any of the aberrant or normal pollen mother-cells. If cytomixis is the cause of their origin it must have occurred at least several cell generations preceding meiosis. Only one anther in a flower has been found with aberrant pollen mother-cells. This limits their origin, in this material, to a state late in ontogeny.

The two contiguous aberrant pollen mother-cells (Plate I, figures 10 and 11) in plant 36-271.11 (2n=42) contained a total of 84 chromosomes: 29 bivalents and 3 univalents, and 10 bivalents and 3 univalents, respectively. It appears that failure of cytokinesis in a spermatogenous cell resulted in one with 84 chromosomes. An atypical premeiotic mitosis then gave rise to the two pollen mother-cells, one with 61 and the other with 23 chromosomes.

Each of the 14 aberrant pollen mother-cells in plant 36-255.5 (2n=41) had 30 chromosomes (11 bivalents and 8 univalents). It is logical to assume than an atypical division occurred at least four cell generations before meiosis and that the sister cell with 11 chromosomes was eliminated.

(The lowest aberrant chromosome number observed in this material was 15 (Plate I, figure 8). Compare also the abortion of the small pollen mother-cells reported by YASUI (1937).)

On the other hand, the aberrant pollen mother-cells in some plants exhibited a wide range with respect to chromosome number (tables 3 and 4). Presumably these owe their origin to one atypical mitosis in each plant, followed by irregularities in succeeding cell generations, or to atypical mitoses of several normal cells. The aberrant pollen mother-cells are usually characterized by relatively large numbers of unpaired chromosomes (tables 2, 3 and 4; Plate I, figures 2, 4, 6, 8 and 9).

Since more than four percent of the 42-chromosome plants contained aberrant pollen mother-cells, their origin in others can not be attributed to an aneuploid condition of the plants. An upset in mitosis seems to be the cause of the origin of these hypo- and hyperploid pollen mother-cells. In hybrid material such as this, atypical mitoses are probably due to mutually incompatible genes contributed by the different parents to some of their progeny, or to incompatible cytoplasm-gene combinations. The fact that the Iumillo×Hope derivatives (which have three species in their parentage) contributed over one-half of the aberrant plants, though including less than one-third of the material examined, emphasizes the rôle of hybridity in producing such incompatibilities. The specificity of the reactions involved is, at the same time, indicated by the complete regularity, so far as examined, of the remaining 320 hybrid plants, of which 184 were unbalanced, aneuploid types.

ACKNOWLEDGMENTS

The writer wishes to express his thanks to Dr. R. F. PETERSON who supplied the seed which made this study possible, and to Dr. C. L. HUSKINS, Department of Genetics, McGill University, for criticisms in the preparation of the manuscript.

SUMMARY

1. Pollen mother-cells with less (and one with more) than 2n chromosomes are recorded in 16 out of 336 plants derived from pentaploid wheat hybrids.

2. The chromosome number in the aberrant pollen mother-cells ranged from 15 to 61; the number of bivalent chromosomes varied from 3 to 29.

3. Their occurrence in only one anther of a plant limits their origin to a stage late in ontogeny.

APPENDIX

Since the above paper was completed, the writer has observed two hypoploid pollen mother-cells with 18 chromosomes (5 bivalents and 8

univalents) in a dwarf, sterile, 40-chromosome (20 bivalents) winter wheat plant. The plant arose in an off-type line of the variety Dawson's Golden Chaff (*T. vulgare*). The conclusion reached earlier is emphasized, namely, that the aberrant pollen mother-cells reported in the above paper are not due to hybridity *per se*, but to incompatible gene combinations in certain hybrid derivatives.

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STUDIES ON SPOTTING PATTERNS. IV. PATTERN VARIATION AND ITS DEVELOPMENTAL SIGNIFICANCE

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Received June 5, 1938

INTRODUCTION

PERHAPS the most striking feature of the spotting patterns found in individuals or strains of many mammalian species is their great variety. They seem all to have the same immediate basis, failure of pigmentogenesis in the hair follicles of some portion of the skin. But the site of the failure is almost unique to each spotted individual.

Considerable variability persists even in strains where the chance of genetic segregation has been minimized by long inbreeding (DUNN 1920). For example, one strain reported in the first paper of this series (DUNN and CHARLES 1937) included individuals with few or no white hairs dorsally, others with unpigmented fur on as much as 25 percent of the dorsum, and a majority of intermediate grade. Among mice with about the same amount of white some had the unpigmented areas only on the left side, some only on the right and some on both.

That there is, however, a certain order in the intra-strain variation of spotting pattern has long been recognized (ALLEN 1914). Thus, in the strain cited above, no individual bore white hairs dorsally elsewhere than on the lumbar region and the distal portions of the legs and tail. Furthermore within the general lumbar area the central portion seems to be more frequently unpigmented than portions either more anterior or more posterior. Similarly there were more mice with white fur only on the feet and tail tip than with white fur extending more proximally on the legs and tail. Thus different body regions are much less sharply differentiated in their non-genetic variation than they are in the single spotted individual, as WRIGHT (1920) has pointed out. Each small skin area has a characteristic potentiality for developing pigment measurable by the proportion of animals within a strain bearing pigmented fur on the area; and the potentiality seems to vary systematically from area to area over the body surface, forming some sort of a gradient field which depends presumably on

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy of Columbia University. The writer is grateful to the Trustees and Council of Columbia University for a University Fellowship in Zoology (1933–4) during tenure of which part of the present work was done; and to Professor L. C. Dunn for many kindnesses during the course of the investigation.

systematic regional differences in the operating conditions of the relevant genes.

It is with the measurement and comparison of such gradient fields, in the presence of six different combinations of "spotting" genes, that the present study is concerned. It has seemed desirable at the same time to re-examine the pigmentogenic enzyme content of pigmented and colorless hair follicles previously studied by ONSLOW (1915) and BLOCH (1917) among others.

MEASUREMENT OF PATTERN VARIATION IN SPOTTED STRAINS AND HYBRIDS

Material

Four strains of mice from the colony of Professor L. C. DUNN, and three groups of hybrids among these strains, have been used. The origin and appearance of these strains, and the genetic basis of their spotting, have been described in detail in Part I (DUNN and CHARLES 1937).

Line K shows white fur on or near the lumbar region, generally in the form of a transverse white belt which may be partial or irregular or contain an "island" of white hairs. Line 190 is similar except that the white areas often seem to extend more posteriorly than any in Line K; there are more individuals with higher proportions of white fur. Line 66 ordinarily has very little white fur, in the form of a narrow unilateral belt or median spot in the lumbar region. Line 19 is "all white" except for a single rump or ear patch on a few individuals. If attention is confined to the proportion of white fur on the dorsal surface, without regard to its location, the four strains and the three groups of hybrids are as follows:

Percent of white on dorsum

Percent of strain showing given amount of white

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	N
wild	100																					—
L66	18	73	7																			162
LK	1	3	18	20	24	27	7															262
L190			1		4	7	11	24	34	16	2											140
LK×L19					3	8	17	23	25	17												60
L66×L19									2	2	16	35	14	10	14	2	2		2	2		57
L190×L19										2	6	8	19	19	25	10	4	6				48
L19																			5	17	78	173

That the intra-strain pattern differences indicated in this table did not depend upon gene differences seems probable from the extent of previous inbreeding and from the mother-offspring, father-offspring correlation coefficients which are L66, 0.22, 0.22; LK, 0.16, 0.09; L190, 0.16, 0.

For convenience the four pure lines will sometimes be referred to in the following pages by their average proportions of white fur; that is, 4%

=L66; 20% =LK; 35% =L190; 100% =L19. The hybrids will be denoted by the product of the parent averages; for example (20% × 100%) =LK × L19.

The 4% and 35% lines each carry the piebald gene "*s*" of the older literature. The 100% line has this gene and in addition at least three other mutant alleles tending to produce white spotting and acting cumulatively with *s*. The 20% strain has the wild type allele of *s* but otherwise carries most or all of the mutant alleles of the 100% line. The main ones of this latter group may be designated by *k, e, f;* it is probable that there are a few other mutant genes with minor effects in the 20% and 100% stocks. The tentative genotypes of the four strains are: 4% *ssKKEEFF;* 20%, *SSkkeeff;* 35%, *ssKKEEff;* 100%, *sskkeeff*, wild type being *SSKKEEFF*. For the present purposes the precise number of genes differentiating the strains is less important than the clear fact that the pattern differences among strains *are* genetically determined (DUNN and CHARLES 1937).

Method

Camera lucida tracings were made of the spotting patterns of the following mice, each at 10± days of age: 27 *ssKkEeff* (35% × 100%); 31 *ssKkEeFf* (4% × 100%); 21 *ssKKEEff* (35%); 48 *Sskkeeff* (20% × 100%); 94 *SSkkeeff* (20%); and 56 *ssKKEEFF* (4%). At the age when tracings were made the hairs are still quite short so that the superficial pattern corresponds closely with that at the surface of the skin.

Each mouse was etherized and so arranged beneath the camera lucida that its projected outline coincided as closely as possible with a standard diagram drawn on quadrille paper with tenth-inch rulings; the projected boundaries between colored and white areas were then traced on the diagram. (It was not always possible to make the two outlines coincide completely; in these cases the pattern boundaries were not traced exactly but were shortened or lengthened proportionately.) There were thus obtained a number of identical-area pattern records, identically subdivided.

Two representative tracings from each of the six genotypic groups are shown in figure 1. (In this figure the original quadrille rulings have not been reproduced and the areas bearing colored fur have been blacked in.)

Data

From the pattern tracings was obtained the "frequency of pigmentation" at each of 508 skin points for each of the six genotypic groups. This was done simply by counting what proportion of the tracings in a group showed pigmented fur at the center of the first square, what proportion at the center of the second square, and so on for each of the 508 squares into which the quadrille rulings divide the standard diagram.

The frequencies so obtained are represented in figures 2–4 where the height of each square pillar is the proportion of mice of the given genotype bearing pigmented fur at the center of the skin region beneath the pillar.

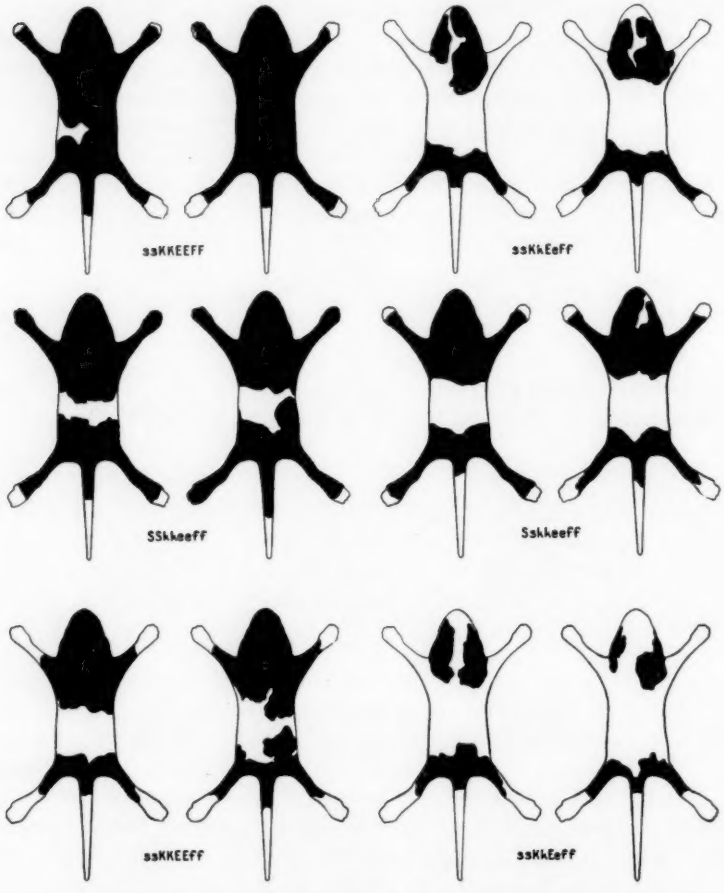

FIGURE 1.—Representative dorsal patterns of six genetic types of spotted mice.

Each figure is a gradient field measuring the regionally-variable potentiality for forming pigment in the presence of a particular gene combination.

The frequencies in figures 2–4 can be identified with "points" of skin only with certain qualifications arising as follows. In tracing the spotting

patterns, as has been indicated, it was not always possible to arrange the mouse to coincide exactly with the outlines of the diagram. In such cases

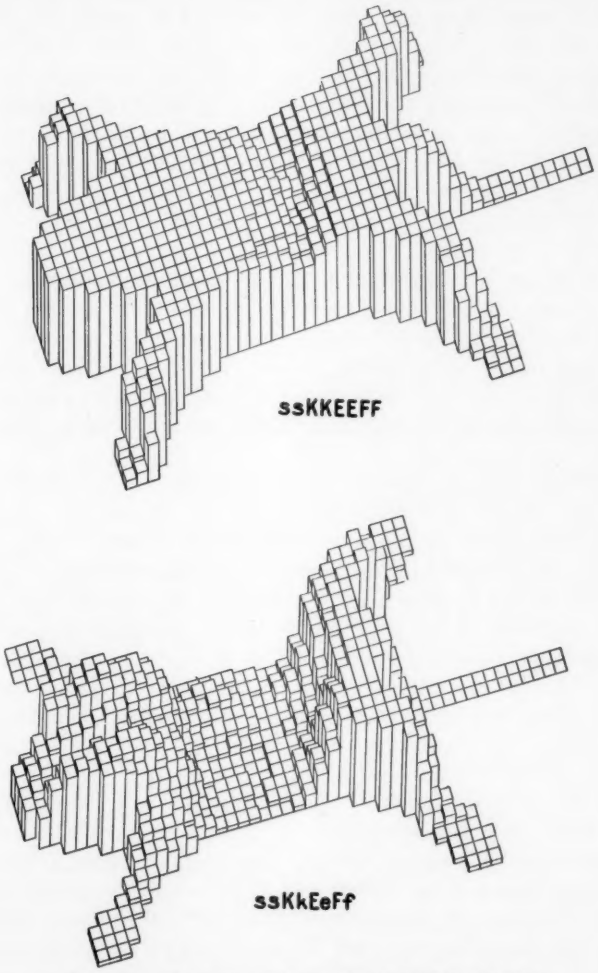

FIGURE 2.—Pigmentation frequencies at 508 skin points in *ssKKEEFF* and *ssKkEeFf* spotted mice.

it was necessary to enlarge or reduce portions of the projected pattern in the tracing process. In addition, slight changes in position of the eye during tracing tended to produce irregular shifts in position of the projected

pattern. In consequence even repeated tracings of the same mouse would be expected to show the same point of skin represented at somewhat different positions on the diagram. Conversely, a constant point of the diagram corresponds to somewhat different points on the different skins traced. The range of this uncertainty can scarcely have been less than 1 percent of the distance from nose tip to tail base, nor much more than 3 percent. Consequently the frequency of pigmented fur represented at a given point in a set of tracings is a complex average of the actual frequencies within an area of diameter equal to 1–3 percent of the body length. For convenience the frequencies will be referred to subsequently as though they described events at actual points of skin, but the limitation may be borne in mind.

The six gradient fields of figures 2–4 are represented as contour maps in figure 5. Each of these maps has been made by plotting on a standard diagram the series of points where the pigmentation frequency of one genotype is 10, 30, 50, 70 and 90 percent; in most cases the points were located by interpolation. The points were then connected by smooth lines, but they give a fair approximation to the contours of the three-dimensional figures.

It will be noticed that several of the maps have contour lines at very nearly the same location. The 30 and 70 percent lines on the head of *ssKkEeff* correspond very closely to the 50 and 90 percent lines, respectively, of *ssKkEeFf*. The 50 percent line on the anterior dorsum of *Sskkeeff* corresponds to the 90 percent line of *SSkkeeff*. Also in general the contour lines on each part of the body run in nearly the same direction for all genotypes, regardless of the actual values. Altogether it seems as though isopotential, that is similarly reacting, strips of tissue were being dealt with: whatever the pigmentation frequency of a particular point may be in the presence of a particular gene combination, all other points on the strip have very nearly the same value.

Two other general relations are readily seen by comparisons among the data of figures 2–4, as follows.

Relative height of frequency field in homozygote and heterozygote. Three such comparisons can be made: *ssKKEEFF* with *ssKkEeFf* (figure 2); *SSkkeeff* with *Sskkeeff* (figure 3) and *ssKKEEff* with *ssKkEeff* (figure 4). In each case the homozygote can be seen to have a higher pigmentation potentiality than the heterozygote at every point (except where both are 0 or 100 percent) as might be expected. That is, substitution of the wild type genes for the mutant alleles simply increases the pigmentation frequency at each point of the skin (except where the frequency is already 100 percent in the heterozygote). As to the magnitude of increment, the most obvious possibility is that it should be everywhere the same. That

such is not the case is shown by table 1: the increment varies with the pigmentation frequency in the heterozygote, and perhaps with the general

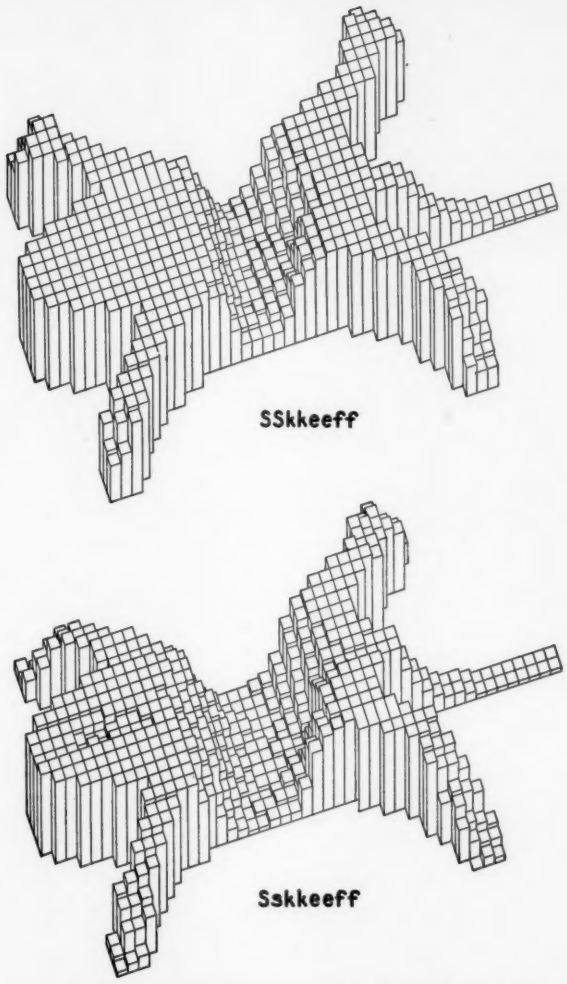

FIGURE 3.—Pigmentation frequencies at 508 skin points in *SSkkeeff* and *Sskkeeff* spotted mice.

region of the body. However, as will be seen in the analytical section, an approach to constancy of increment from heterozygote to homozygote is obtained if the pigmentation frequencies are transformed in a simple way.

Action of different loci on the frequency field. The simplest expectation here is that the six gradient fields should have the same general form and fall into a simple series with respect to height, their order being the same at every point of the skin. Such a situation might be expected if the *s*, *k*,

TABLE 1

Pigmentation frequency, in percent, of corresponding skin points in heterozygous and homozygous spotted mice; the increments from heterozygote to homozygote are shown in brackets.

Sskkeeff (56 MICE)		AVERAGE FREQUENCY IN *SSkkeeff* (94 MICE)					
RANGE	AVERAGE	HIND LEGS AND TAIL	POSTERIOR DORSUM	ANTERIOR DORSUM	FORELEGS	HEAD	TOTAL POINTS
1– 9	5	18 (13)	34 (29)	25 (20)	47 (42)	—	48
10–18	14	30 (16)	42 (28)	38 (24)	55 (41)	—	42
19–26	22	49 (27)	58 (36)	61 (39)	79 (57)	—	19
27–34	30	70 (40)	74 (44)	67 (37)	92 (62)	—	12
35–43	39	86 (47)	84 (45)	84 (45)	79 (40)	—	10
44–51	47	79 (32)	—	93 (46)	97 (50)	—	8
52–59	55	97 (42)	95 (40)	96 (41)	97 (42)	—	13
60–68	64	95 (31)	95 (31)	100 (36)	97 (33)	—	9
69–76	72	100 (28)	95 (23)	100 (28)	97 (25)	—	17
77–84	80	100 (20)	98 (18)	100 (20)	98 (18)	—	29
85–93	89	100 (11)	98 (9)	100 (11)	100 (11)	—	17
94–99	96	98 (2)	100 (4)	100 (4)	100 (4)	—	46
100	—	100	—	100	100	—	79
ssKkEeff (27 MICE)		AVERAGE FREQUENCY IN *ssKKEEff* (21 MICE)					
0	—	9	4	27	41	—	137
1– 9	5	9 (4)	6 (1)	48 (43)	42 (37)	—	98
10–17	13	33 (20)	32 (19)	73 (60)	64 (51)	96 (83)	34
18–24	21	36 (15)	40 (19)	76 (55)	74 (53)	94 (73)	23
25–32	29	—	59 (30)	85 (56)	—	100 (71)	12
33–39	36	—	83 (47)	89 (53)	—	100 (64)	15
40–47	44	55 (11)	78 (34)	93 (49)	—	99 (55)	28
48–54	51	—	77 (26)	93 (42)	—	100 (49)	14
55–62	58	51 (–7)	95 (37)	95 (37)	—	100 (42)	17
63–69	66	71 (5)	—	100 (34)	—	100 (34)	10
70–77	73	82 (9)	92 (19)	—	—	100 (27)	15
78–84	81	96 (15)	94 (13)	—	—	100 (19)	23
85–91	88	97 (9)	100 (12)	—	—	100 (12)	20
92–99	95	98 (3)	100 (5)	—	—	100 (5)	18
100		—	100	100	—	100	20

e and *f* alleles all had the same sort of primary effect, but different degrees of activity, as WRIGHT (1920) has assumed to be the case for a group of spotting genes in the guinea pig. The expectation seems to be fulfilled in the case of *ssKKEEFF* (4%), *ssKKEEff* (35%), *ssKkEeFf* (4%×100%) and *ssKkEeff* (35%×100%). Six comparisons can be made among these genotypes, taking two at a time: *ssKKEEFF* and *ssKKEEff* have already

been seen to be consistently higher than *ssKkEeFf* and *ssKkEeff*, respectively; direct comparison of figures 2 and 4 shows that *ssKKEEFF* is

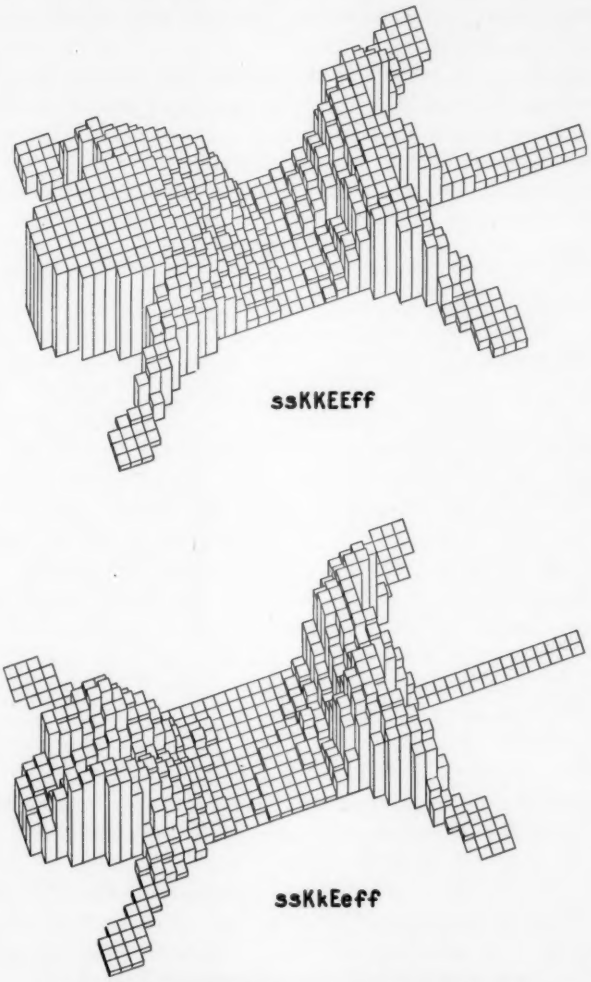

FIGURE 4.—Pigmentation frequencies at 508 skin points in *ssKKEEff* and *ssKkEeff* spotted mice.

higher than *ssKkEeff*, *ssKKEEff* higher than *ssKkEeFf*. Two comparisons remain to be made: *ssKKEEFF* (4%) with *ssKKEEff* (35%) and *ssKkEeFf* (4%×100%) with *ssKkEeff* (35%×100%); these are shown

in table 2. The *ssKKEEFF* field is higher than that of *ssKKEEff*, or equal to it at 100 percent, except at 42 of the 508 points. Nearly all of these points are on the extreme distal portions of the hind legs and tail where the data are least reliable since only few animals of either genotype bear pigmented fur there.

The same sort of relation is found between the frequency fields of *ssKkEeFf* and *ssKkEeff*. The former is equal to, or greater than, the latter at 431 of the 495 points recorded in table 2. Of the remaining 64 points, 48 bore pigmented fur in none, or only one, of the 31 *ssKkEeFf* mice and in one, or two, of the 27 *ssKkEeff* mice; the other 16 points had very nearly

TABLE 2

Relative frequency of pigmentation at corresponding skin points of different spotting genotypes.

RELATIVE FREQUENCY	NUMBER OF POINTS					
	HIND LEGS AND TAIL	POSTERIOR DORSUM	ANTERIOR DORSUM	FORELEGS	HEAD	TOTAL POINTS
ssKKEEFF>*ssKKEEff*	55	86	117	59	6	323
ssKKEEFF<*ssKKEEff*	39	0	0	3	0	42
ssKKEEFF=*ssKKEEff* =100%	24	28	3	0	88	147
ssKkEeFf>*ssKkEeff*	83	80	95	34	80	372
ssKkEeFf<*ssKkEeff*	2	20	11	17	14	64
ssKkEeFf=*ssKkEeff* =0% or 100%	30	11	7	11	0	59
ssKKEEff>*Sskkeeff*	22	8	44	2	29	105
ssKKEEff<*Sskkeeff*	68	80	73	50	6	277
ssKKEEff=*Sskkeeff* =100%	24	26	3	0	59	112

equal pigmentation frequencies. Since the differences at these discordant points are so small, they may perhaps be only a consequence of sampling error.

Thus it seems probable that the frequency fields of *ssKKEEFF*, *ssKKEEff*, *ssKkEeFf* and *ssKkEeff* fall into a consistently diminishing series in the order indicated and that the *KE* and *F* wild type genes may thus have the same sort of primary or intermediate action.

There seems however to be a different relation between the genotypes containing *KE* without *S* and those containing *S* without *KE*. The most extensive comparison available is between *ssKKEEff* (35%) and *Sskkeeff* (20%×100%), in table 2. The significant regions here are the head, where *Sskkeeff* often shows a white blaze although *ssKKEEff* never does,

and the posterior dorsum, where *Sskkeeff* has consistently higher pigmentation frequencies. A similar reversal in relative height of fields can be seen by comparing figures 2 and 3; over the general lumbar area, *ssKKEEFF* has much higher pigmentation frequencies than *SSkkeeff*; but on the distal halves of the legs *SSkkeeff* is higher. It seems very unlikely, from the sizes of the two samples and from the range of frequencies over which the reversal is observed, that the reversal is only a matter of sampling error.

SSkkeeff (20%) and *Sskkeeff* (20% × 100%) thus do not fit into a consistent series with the other four genotypes, but have a pigmentation frequency field of fundamentally different shape. It seems probable accordingly that *S* has a different primary or intermediate action than *K*, *E* and *F*.

Correlations in pattern variation. A fourth general phenomenon of the variation in spotting pattern is the interrelation in pigmentation response (that is, forming or not forming pigment) among large groups of skin points. This has been previously studied by ALLEN (1914) in genetically heterogeneous groups from various mammalian species and by ILJIN (1928) in guinea pigs. The immediately observable fact is that the skin of any spotted animal (except in the case of dominant spotting) consists of a small number of large pigmented or unpigmented regions. Furthermore there is a limited variation in the arrangement of the two kinds of regions, within any homogeneous stock. In the lighter genotypes (*ssKkEeFf*, *ssKkEeff*) there are typically three patches of colored fur dorsally: one covering both sides of the rump and extending somewhat onto the legs and tail; one centering on each ear and extending towards the eyes and scapula. The two ear patches may be confluent; there may be an "island" of colored fur somewhere between the ear and rump patches. In the darker genotypes (*ssKKEEFF*, *SSkkeeff*) there are rarely more than six regions of white fur, dorsally; one on each foot, one on the tail tip and one on the lumbar area. The foot and tail patches may extend for various distances centrally; there are occasionally two lumbar patches; in *SSkkeeff* there is sometimes a white blaze on the forehead. In all groups no part of the leg or tail is pigmented unless there are also pigmented hairs on all more proximal parts of the appendage at least as far as the point indicated by the intersection of the dotted lines from leg and tail in the *ssKKEEFF* contour map in figure 5. Conversely if the knee, or tail base, bears *white* hairs, all more distal parts of the appendage do also. No part of the foreleg is pigmented unless there are pigmented hairs as far centrally as the base of the ear (intersection of the dotted lines on arm and body proper in figure 5). The anterior tip of the head is never pigmented unless there is colored fur at least as far posteriorly as the rear of the eye.

Although leg and tail pigmentation seem thus to depend on the formation of pigment more centrally they do not depend on each other. That is (in *SSkkeeff*, *Sskkeeff* and *ssKKEEFF*) the proportion of animals with pigment at any one point on the leg and any one point on the tail has not

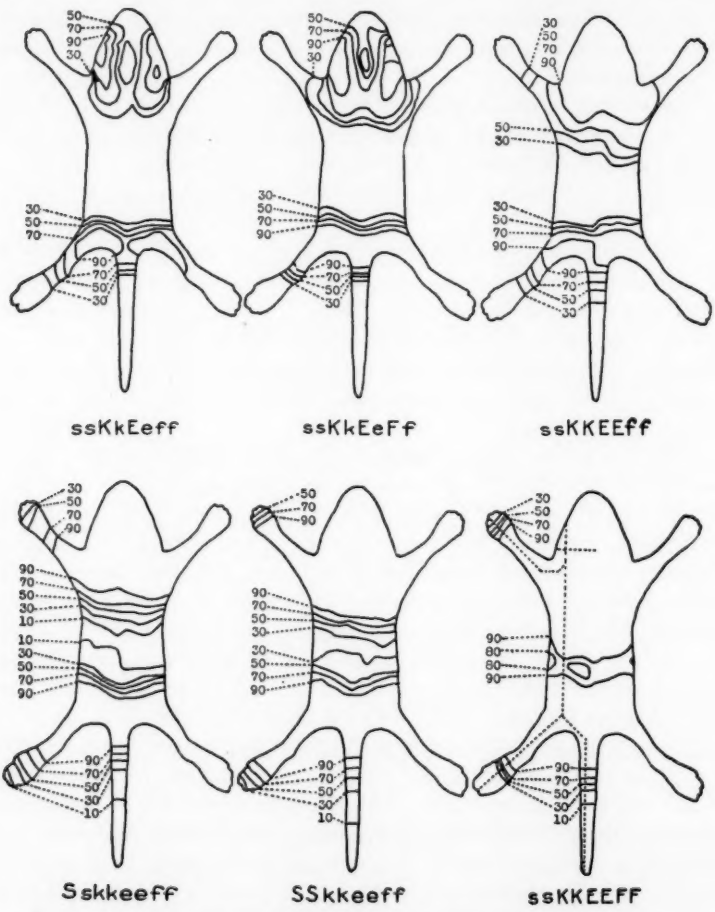

FIGURE 5.—Contours of the pigmentation frequency surfaces in figures 2–4.

been found to be significantly different from the chance expectation, that is, from the product of the pigmentation frequencies of the two points. Similarly no relation has been found between pigmentation of points on the tail and foreleg, on hind and fore leg or on opposite sides of the body.

A somewhat different situation is found in the central two-thirds of the body proper, that is, the central two-thirds of the longitudinal dotted line on the *ssKKEEFF* contour map in figure 5. The coefficients of association in pigmentation at pairs among ten points along this axis are shown in table 3, for *SSkkeeff* which is the only group of tracings large enough to give fairly reliable results. There is a consistent association in presence or absence of pigment between any pair of adjacent points and

TABLE 3

Association in presence or absence of pigment at ten equidistant points extending from shoulder to rump (along the dotted line in figure 5) in SSkkeeff spotted mice. N_{ij} is the number of animals with pigment at each of two points; N_i with pigment at the more anterior point only; N_j, at the most posterior point only; N_0, at neither; c is the coefficient of association, $(N_{ij} \cdot N_0 - N_i \cdot N_j)/(N_{ij} \cdot N_0 + N_i \cdot N_j)$.

POINTS	N_{ij}	N_i	N_j	N_0	c	POINTS	N_{ij}	N_i	N_j	N_0	c
1.2	52	30	0	12	1	3.9	17	6	59	12	−0.27
1.3	23	59	0	12	1	3.10	21	2	69	2	−0.53
1.4	21	61	2	10	0.27	4.5	19	4	5	66	0.97
1.5	23	59	1	11	0.62	4.6	17	6	14	57	0.84
1.6	28	54	3	9	0.22	4.7	16	7	22	49	0.67
1.7	31	51	7	5	−0.39	4.8	17	6	33	38	0.53
1.8	41	41	9	3	−0.50	4.9	17	6	59	12	−0.27
1.9	65	17	11	1	−0.48	4.10	20	3	70	1	−0.83
1.10	78	4	12	0	−1	5.6	21	3	10	60	0.95
2.3	21	31	2	40	0.86	5.7	19	5	19	51	0.82
2.4	14	38	9	33	0.15	5.8	20	4	30	40	0.74
2.5	14	38	10	32	0.08	5.9	20	4	56	14	0.11
2.6	17	35	14	28	−0.01	5.10	21	3	69	1	−0.82
2.7	17	35	21	21	−0.35	6.7	28	3	10	53	0.96
2.8	24	38	26	16	−0.31	6.8	28	3	22	41	0.94
2.9	40	12	36	6	−0.29	6.9	27	4	49	14	0.32
2.10	50	2	40	2	0.11	6.10	27	4	63	0	−1
3.4	16	7	7	64	0.91	7.8	32	6	18	38	0.84
3.5	12	11	12	59	0.69	7.9	36	2	40	16	0.74
3.6	13	10	18	53	0.59	7.10	36	2	54	2	−0.20
3.7	12	11	26	45	0.31	8.9	43	3	29	15	0.78
3.8	15	8	35	36	0.31	9.10	74	2	16	2	0.64

a decreasing association between points successively further apart. From each point the association falls to zero and then assumes negative values.

The meaning of these negative values is not clear, but they must arise in part from the tracing method. The inaccuracies of alignment of the projected outline of a mouse against a standard diagram are such that, if a given mouse were traced repeatedly, the boundaries of a given white spot would not lie identically on the tracing. In general where the anterior boundary of the spot has been traced too far forward, the posterior boundary will also be too anterior; where the front boundary has been traced

further back, the rear boundary will also be relatively posterior. In repeated tracings of the same mouse there should thus be an association coefficient of −1 between presence of pigment at one point and another separated from it by the length of the spot traced. In tracings of different mice there will be a tendency to similar negative association between any two points which may lie immediately fore and aft of a white spot. Because of this error it is difficult to attach any meaning to the negative coefficients in table 3; the positive values indicate a real association.

In general, the formation of pigment on either the posterior or anterior dorsum shows a decreasing association with pigment on points successively nearer the opposite end of the body. Formation of pigment in the middorsum is decreasingly associated with the pigmentation response of points successively further away either anteriorly or posteriorly. It seems probable that a similar relation holds for all six genotypes.

ANALYTICAL

Pigmentation frequencies. WRIGHT (1920, 1936) has used an "inverse probability transformation" to convert the proportion of white fur in the skin of a spotted guinea pig into a quantity which is equivalent to an average "concentration" of some material throughout the skin, relative to a "critical concentration" for pigment formation. The same transformation may be used to convert the pigmentation frequency of one skin point within a genotype into an "average concentration" at that point. The basis of the transformation is as follows.

"Although the skin of a piebald guinea pig is divided sharply into areas in which pigment is either produced to the full amount characteristic of the animal, or is wholly absent, it is not to be supposed that the influences, which at some critical period in ontogeny determine whether a region is to be colored or white, are so sharply alternative in themselves. It seems more reasonable to suppose that the sums of favorable and unfavorable influences in different parts of the skin could be arranged in a graded series. Doubtless in certain white regions a slight difference in the conditions would have enabled color to develop, while in others a great change would have been necessary. Similarly with colored areas. Suppose, then, that the skin is divided into a large number of equal areas and that it were possible to determine the true potentiality of each area at the critical period in development. . . . All areas which exceed this a 'critical potential' produce color, while those which fall below, however slightly, remain white." (WRIGHT 1920.)

The "true potentiality" of the cells ancestral to a small area *of a particular skin* at the "critical period in ontogeny" might be thought of, in the simplest possible way, as a concentration P of some unknown material.

The "critical potential" would then be the smallest concentration P_0 which could bring about the ultimate formation of pigment. Pigmented hairs would be borne on an area only if P had been equal to, or greater than, P_0. Since some regions of an individual bear pigmented fur, others white fur, either P or P_0 or both must vary from region to region of the same skin. Since the same small skin area may have pigmented fur in some individuals and white fur in other individuals of the same genotype, either P or P_0, or both, of that area must vary somewhat from animal to animal, P being the greater in some, P_0 in others. Since the spotting patterns are at least roughly similar among animals of the same genotype, the regional variation in P or P_0 must have the same general form throughout the group; that is, both P and P_0 must tend to a modal value for each skin

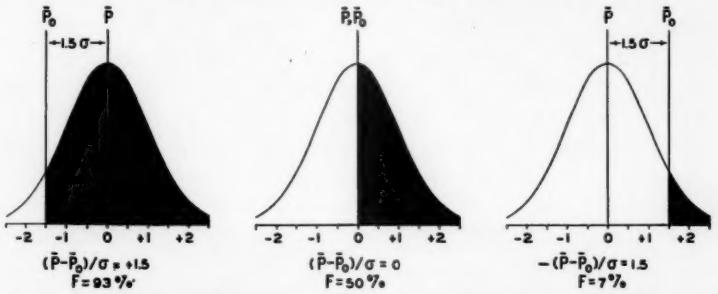

FIGURE 6.—Relation of pigmentation frequency (F, shaded area) at a skin point to average difference between a variable "concentration" P at that point and a minimal "concentration" P_0 for pigment formation (modified from Wright 1920).

point among individuals of the same genotype. Let us assume that the frequencies of various values of the difference $(P-P_0)$ for a given small skin area would form a normal distribution of standard deviation σ; the average value of the difference may be represented by $(\overline{P}-\overline{P}_0)$. The pigmentation frequency F to be expected for any value of $(\overline{P}-\overline{P}_0)$, relative to σ, can be found from a table of areas under the normal curve. That is, F is the probability function of $(\overline{P}-\overline{P}_0)/\sigma$. Three examples are shown in figure 6.

Conversely, the value of $(\overline{P}-\overline{P}_0)/\sigma$ corresponding to a particular F can be found from a table of the normal curve areas; that is, $(\overline{P}-\overline{P}_0)/\sigma$ is the inverse probability function of F. A few pairs of values are as follows:

F (in %)=	1	2	5	10	20	50	80	90	95	98	99
$(\overline{P}-\overline{P}_0)/\sigma=$	−2.32	−2.06	−1.64	−1.28	−0.84	0	+0.84	+1.28	+1.64	+2.06	+2.32

This inverse probability transformation might be applied to each of the values in figures 2–4. No additional information would be gained, though: the relative steepness of various parts of the surfaces would be changed, but the order of their height, and the regional reversal of height between

Skef and *sKEF* genotypes, would not be altered. Insofar, however, as $(\overline{P}-\overline{P}_0)/\sigma$ may be thought of as a quantity like a difference of two concentrations, it is closer than a pigmentation frequency to the sort of variable on which gene substitution might be expected to have an equal additive effect throughout the skin. If this were so, the $(\overline{P}-\overline{P}_0)/\sigma$ values

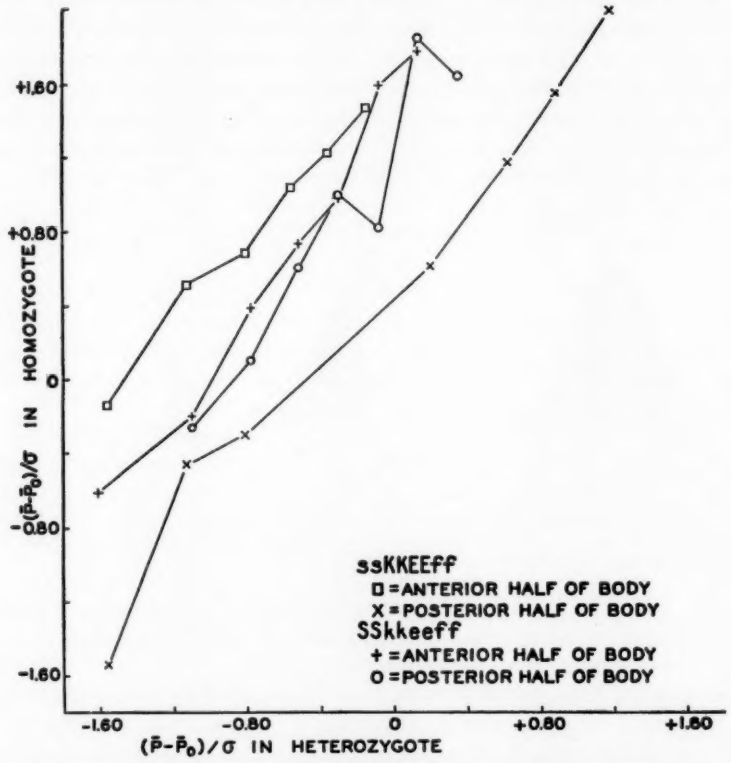

FIGURE 7.—Relation between transformed pigmentation frequencies of a heterozygous spotted type and the corresponding homozygote.

for a series of points in the homozygote, plotted against the values for the same points in the corresponding heterozygote, ought to fall along a straight line of slope 1. The transformed data of table 1 have been plotted in this way in figure 7 (the values for forelegs and anterior dorsum have been grouped together, as have the values for hind legs and posterior dorsum).

Three general relations seem to be indicated by figure 7:

(1) Within either the anterior or posterior half of the body the $(\overline{P}-\overline{P}_0)/\sigma$

values of *ssKKEEff* (35%) do fall along a straight line of slope 1 against the corresponding values in *ssKkEeff* (35%×100%). That is, there is a constant increment from heterozygote to homozygote as though *K* and *E* had a simply additive effect; but

(2) The constant increment within the anterior half of the body is much greater than the increment within the posterior half; the values are approximately 1.6 and 0.6. (There is some indication of an even higher increment on the head proper than on the forelegs and anterior dorsum.)

(3) The $(\overline{P}-\overline{P}_0)/\sigma$ values of *SSkkeeff* (20%) fall along approximately the same straight line against the *Sskkeeff* (20%×100%) values for both halves of the body, but the line seems to have a slope greater than 1. This is as though *S* had some sort of multiplicative effect. Here again a difference in nature of primary action between *S* and *KE* is indicated.

Correlations. From a comparative study of spotted mammals and birds ALLEN (1914) concluded that in both classes the skin consists dorsally of eleven contiguous or slightly overlapping cell groups within each of which, at some stage of development, a substance or process necessary for subsequent pigment formation spreads from the center towards the edges; that the extension occurs independently in the different areas and to its full extent in animals which become unspotted; that where the extending process stops short of the edge of its area, the peripheral territory becomes unpigmented; and that the spotting pattern of an individual is identical with the extent of spread of the determining process from the centers of the eye, ear, scapular, lumbar and rump areas of each half of the body.

ILJIN (1928) reached a different conclusion primarily from correlation studies on spotted guinea pigs: that spotting patterns are determined, not by restriction of a process which occurs in unspotted animals, but by extension of an action found only in spotted individuals; that in the mouse this action spreads from each of twelve "points of depigmentation" on the dorsal surface; and that between some of these points there is a correlation in degree of extension, the magnitude of correlation depending on the distance between the points considered.

ILJIN's "points of origin of depigmentation" coincide very closely with the margins of, or boundaries between, ALLEN's pigmentation areas. Both are located, in the main, at the common low points of the present pigmentation frequency fields, that is on the toes and tail tip, nose tip, center of forehead and anterior lumbar region.

ILJIN assumed depigmentation centers at front and rear of the third-quarter of the dorsum; ALLEN, a pigmentation center in about the middle of this quadrant. Now let two points be considered, one near the front, one near the rear of the third quadrant. If both points bear unpigmented fur in a given mouse it is indicated, according to ILJIN, that one has not

been reached by a process spreading from the anterior center, the other by a process from the posterior center. According to ALLEN, neither has been reached by one process diffusing from a single center. Now this quadrant of the dorsum corresponds to the series of points from 5 through 10 in table 3 among which most pairs of points show a reasonably large pigmentation association. These associations would mean peripheral correlation within one spreading process according to ALLEN, correlation between two processes under ILJIN's scheme. The former is at least simpler, though not necessarily more probable; it is equivalent to supposing that any one extending process tends to spread nearly equally in all directions; that the variation in extent is like the variation in aperture of an iris diaphragm rather than like an irregular, amoeboid variation in outline.

But ALLEN's scheme meets a certain amount of difficulty in the data of table 3. Point 9 has been shown never to be pigmented without pigmented fur extending from there to the posterior margin of the rump, which would mean that the last quarter of the dorsum lies in the rump field. But the pigmentation response of point 9 is positively associated also with that of points as far forward as the center of the dorsum (point 5), which would seem to mean that points 5 and 9 lie in ALLEN's lumbar field. The same difficulty is met at point 2, which seems to lie in both scapular and lumbar fields.

One possible solution is suggested by those skins which show "islands" or "peninsulas" of pigmented fur surrounded by colorless fur (figure 1). Under ALLEN's hypothesis these are cases where the spreading processes of three adjacent fields have failed to meet at their two borders. They may be found in three general positions centering approximately at points 1, 5 and 9 of table 3; that is, at the anterior end of the second quadrant, between second and third quadrants, and at the posterior end of the third quadrant. The three types of island are not sharply demarcated, but they do have a modal tendency. The first and third correspond to restriction of ALLEN's shoulder and lumbar fields, respectively. But the second is at the boundary between these two fields, and so might be supposed to define another pigmentation center ("mid-dorsal") not indicated by ALLEN, covering part of the territory of his scapular and lumbar areas. Within this common region, it might be supposed that pigmentation is sometimes determined by the spreading process from one center, sometimes by that of the other center, or perhaps by both jointly. One further alteration in ALLEN's scheme would be necessary from the present data. Since pigmentation on the foreleg does not occur without pigment on the ear, the former must belong to the ear field, rather than to the scapular as ALLEN supposed. Thus, if overlapping local fields are involved at some stage in the

embryogeny of spotting pattern, they perhaps number six on each side of the body: eye, ear, scapular, mid-dorsal, lumbar and rump.

In the absence of any direct demonstration the pigmentation fields of ALLEN and ILJIN, inferred from the regularities in location of pigmented and white fur areas, and the "concentration gradients," inferred from the regional variation in pigmentation frequency, are both merely ways of restating the primary data of genetic and non-genetic variation in spotting patterns. Each suggests that the next step in the analysis of the developmental basis of spotting patterns is a search for conditions which vary systematically thoughout the embryonic skin (and also to a certain extent from individual to individual), for example, time of attaining some particular stage of morphological differentiation, rate of growth, etc. Some such pattern seems almost certainly involved, determining that in some regions of the skin cells containing several mutant (perhaps inactive) genes can form pigment whereas in other regions of the skin the whole wild-type gene set must be present. Regarded in this way the formation of mammalian spotting patterns is a relatively superficial and accessible analogue to the more fundamental ontogenetic processes by which genes and cytoplasmic differentials, tracing back ultimately to the structure of the fertilized egg, together determine the location of morphological elements more important than hair pigment. At least from this aspect they deserve further study.

THE PHYSIOLOGICAL DIFFERENCE BETWEEN COLORED AND WHITE AREAS

The question has frequently arisen what component of the reaction system leading to presence of pigment varies in the skin of a spotted mammal. ONSLOW (1915) and BLOCH (1927) each reported the white-furred skin regions of spotted mammals to lack a melanogenic enzyme which is present in the colored-furred areas and throughout the skin of unspotted animals. ONSLOW identified the enzyme as tyrosinase (rabbits, mice); BLOCH, as a more specific "dopa oxidase" (guinea pigs). The latter has since come to be rather generally accepted as the melanogenic enzyme of mammals.

PUGH (1933) reported that tyrosinase can only seldom be found even in extracts of all-black skin. In about 15 experiments she found no evidence of tyrosinase activity even when ONSLOW'S procedure was followed as closely as possible. But in a further set of five extractions, two did produce a grey coloration in tyrosine solution after 30–33 hours. She concluded that black rabbit skin does contain a true tyrosinase.

BLOCH (1917, 1927) reported that frozen sections of skin, immersed in a 0.1% solution of dioxyphenylalanine pH 7.3–7.4, in 6–12 hours show formation of additional pigment granules *only* in those places where

melanin had previously formed naturally—in the melanoblasts. Among a number of mono- and di-hydric phenols only dioxyphenylalanine gave the reaction. On this basis BLOCH concluded that mammals, unlike great numbers of invertebrate and plant species, do not contain a tyrosinase but have another pigment forming enzyme which is substrate specific to dioxyphenylalanine.

BLOCH found the dopa-oxidase reaction of sections of white-spotted guinea pig skin to be negative in the follicles producing white hairs and positive in those forming pigmented hairs. But PRZIBRAM, DEMBOWSKY and BRECHER (1921) have suggested, from evidence that regions in active melanogenesis are more alkaline than the surrounding tissue, that the dopa reaction is identical with the spontaneous oxidation which dopa undergoes in vitro at alkalinities not much great than pH 7.5.

Hence so far as can be told from the published material, there exists an anomalous situation with respect to the physiological differences between colorless and pigment-forming follicles of spotted mammals: an enzyme difference is reported by each of two experimental methods, but doubt is cast on the validity of each. If PUGH's finding is correct that even with ONSLOW's extraction method only about two out of seventeen samples from black skin show tyrosinase activity, then ONSLOW's results may have been accidental; if (as is not clear from his report) he tested only one or two white skin extracts, the failure of tyrosinase activity in these cases may have been only an instance of the same random failure which PUGH observed even in black extracts. And BLOCH's report of a dopa-oxidase difference between colored and unpigmented follicles in frozen sections may have been non-enzymic depending only upon the reported greater alkalinity of the pigment-producing follicles.

Accordingly it has seemed desirable to re-examine the existence of an enzyme difference between colored- and white-furred areas of piebald mouse skin. Because regular results could not be secured in preliminary tests with skin extracts and dopa, tyrosinase was used as a substrate in further study which was carried out as follows.

Comparable tests were made of the tyrosinase activity of extracts of skin of black unspotted mice and of extreme spotted, "all white," mice (DUNN and CHARLES 1937) only few of which have any pigmented hairs. Groups of 8–12 animals 2–4 days old were decapitated and bled as thoroughly as possible. The skins were removed with minimal connective tissue adhering and ground in a glass mortar with sand, chloroform, water or Ringer solution and pH 7.4 phosphate buffer (the amounts are shown in table 4). The resulting fairly homogeneous, gelatinous, grey or pale pink paste was centrifuged and filtered through a Buechner funnel. The opalescent filtrate was divided among a number of test tubes to which were

TABLE 4

Preparation of skin extracts for tyrosinase tests.

EXTRACT NUMBER	COLOR OF SKIN	NUMBER OF SKINS	EXTRACTION IN			
			pH 8 BUFFER	WATER	RINGER	$CHCl_3$
I.	black	8	1.5 cc.	—	3.5 cc.	0.5 cc.
II.	albino	8	1.5 cc.	—	3.5 cc.	0.5 cc.
III.	black	11	1.5 cc.	—	3.5 cc.	0.5 cc.
IV.	black	10	1.5 cc.	—	3.5 cc.	0.5 cc.
V.	black	12	5.0 cc.	—	—	5.0 cc.
VI.	white*	12	5.0 cc.	—	—	5.0 cc.
VII.	black	9	2.0 cc.	3.0 cc.	—	few drops
VIII.	white*	9	2.0 cc.	3.0 cc.	—	few drops

* "All white" extreme spotted.

added substrate as shown in table 5 and a few drops of chloroform. Tubes were kept either at room temperature or at 38°C. as indicated, and observed after intervals varying from 12–24 hours. The results are shown in table 5.

In the first series tyrosinase activity, without peroxide and so meeting the criteria for a true tyrosinase, was found in the extracts of two out of three groups of black skins (I and III). The degree of melanization after 20

TABLE 5

Tyrosinase activity of skin extracts.

EXTRACT NUMBER	COLOR	TEMP. (°C.)	TIME (HRS.)	1 CC. H^2O	1 CC. DOPA 0.04%	1 CC. TYRAMINE 2.02%	1 CC. TYROSINE 0.04%	
H^2O	—	20±	17	—	++	—	—	
I.	black	20±	17	—	+++*	—	+++	
II.	albino	20±	48	—	+++	—	—	
III.	black	38	19	—	+++	—	+++	
III. (diluted 1:5 with water)	black	38	19	—	+++	—	—	
IV.	black	20±	21	—	+++	—	—	
IV.	black	20±	21	—	++ p-cresol catechol aniline†	— p-cresol catechol	— p-cresol aniline	p-cresol
V.	black	20±	24		++‡	+	+	—
VI.	white	20±	24		—	—	—	—
VII.	black	20±	20		++	+	—	—
VIII.	white	20±	20		—	—	—	—

* +++ indicates deep gray color through top half of solution when tyrosine is substrate, throughout when dopa is used.

† 1 cc. 0.1% p-cresol; 0.1 cc. 0.1% catechol; 0.5 cc. 1% aniline.

‡ ++ indicates medium brown color; +, reddish tan.

hours was considerably less than that reported by ONSLOW who found a charcoal black ring at the top of his test solutions, but was comparable to that secured by PUGH.

A third extract (IV) of black skin did not give any evidence of tyrosinase activity, either full strength or diluted. This same inactivity was found in extract III diluted 1:5 with water, although the undiluted extract was active. The fact that in this case at least dilution led to loss of tyrosinase activity suggests that in IV, where the method was apparently comparable some unknown difference in procedure led to lower concentration of enzyme in the extract as PUGH suggested in reference to the irregularity of her results. It is of interest in this connection that PUGH (1933) reported the same unexplained effect of dilution on the tyrosinase activity of mealworm extracts.

The difficulty in using dopa, under simple conditions, as a substrate for testing the presence of melanogenic enzymes in skin extracts is indicated in table 5 by its reaction in all cases, even without addition of other substances.

In an attempt to minimize the number of reactions involved in the tyrosinase tests, p-cresol was tried as a substrate known to be oxidized by tyrosinase without undergoing the complex series of secondary reactions which occur when tyrosine is used. As a possible means to increase the sensitivity of these further tests catechol and/or aniline were also added, in view of RICHTER'S report (1934) that (1) the direct action of tyrosinase is upon o-dihydric and not monohydric phenols, forming o-quinones, and that monohydric phenols serve as substrates for tyrosinase only after reacting with o-quinone to form dihydric phenols: and (2) that higher concentrations of o-quinone inhibit the enzyme. Hence catechol was added as a priming agent (that is, to supply o-quinone) in concentration so low that its own oxidation would not add enough color to the solution to suggest tyrosinase activity. This concentration should still be high enough to furnish adequate substrate for the initial action of the enzyme, if present. The aniline was added to combine with any possible, though unlikely, excess of o-quinone which might be formed.

Two black skin extracts tested under these conditions both showed the presence of tyrosinase (V and VII), with and without aniline. When catechol was omitted, only one of the extracts showed activity presumably because of the lack of enough priming material like catechol in the skin extract.

Two extracts from the skin of extreme spotted animals (VI and VIII), each tested simultaneously with one of the black extracts, did not show evidence of tyrosinase activity under any test conditions.

Since only one out of the total of five black extracts tested failed to react

positively for tyrosinase, the chance that two samples from other skin of similar enzyme content should both fail for the same unknown reason as the black is only about one in twenty-five (that is, the product of the chances that one should fail). Hence it may be concluded that probably, as ONSLOW reported originally, the white-furred areas of piebald mouse skin either lack, or have a much lower concentration of, the melanogenic enzyme present in the skin of colored animals. The data definitely confirm PUGH'S finding that the enzyme is a true tyrosinase in that is operates without added peroxide and that, contrary to BLOCH'S conclusion, mammals (or at least mice) thus do not lack the potentially melanogenic enzyme present in invertebrates. That this tyrosinase lies at least partly in the melanoblasts of the hair follicles and is actually the melanogenic enzyme of those cells is not proved by the present results, although it seems likely from the probable deficiency of the enzyme in the regions of skin where the follicles are not producing melanin. It is also yet to be proved that the colored-furred areas of spotted mouse skin have the same concentration of tyrosinase as the skin of unspotted mice, although this seems likely from the identity of the pigmentation. And it remains uncertain whether the white-furred areas of spotted mice are completely lacking in tyrosinase.

SUMMARY

The patterns of white-spotted mice have long been known to be rather diverse, even within presumably almost isogenic strains. The variability is limited, however; some regions of the skin bear pigmented fur in every individual; other regions always bear white fur, and it is only the intervening areas that have a variable behavior which may be characterized by the proportion of cases in which the hairs are pigmented.

1. Counts have been made of the proportion of animals bearing pigmented fur on each of 508 very small skin areas, in the presence of each of six combinations of "spotting" genes.

2. The proportions so obtained ("pigmentation frequencies") vary systematically over the skin surface, in the presence of any one genotype, forming a gradient field which drops off from the ear and rump regions towards the feet, tail tip, and mid-lumbar region.

3. The frequency fields of *ssKKEEFF* (4% of white fur), *ssKKEEff* (35%), *ssKkEeFf* (hybrid between 4% and 100% white strains) and *ssKkEeff* (35%×100%) are found to have the same general form but consistently different heights, the frequencies diminishing in the order indicated.

4. Similarly the frequency field of *SSkkeeff* (20%) is consistently higher than that of *Sskkeeff* (20%×100%); but neither of these two falls into the

previous series; that is, both have a somewhat different form than the fields obtained in the presence of *ss*, lower in the anterior dorsum and head, higher on the posterior dorsum and legs.

5. The difference in shape of pigmentation frequency field between genotypes containing *KEF* without *S* and those containing *S* without *KEF* is taken to indicate that *KEF* and *S*, though both acting ultimately on pigmentation frequency, have different sorts of primary effect.

6. The increase in pigmentation frequency of a given point from heterozygote to homozygote (*Sskkeeff* to *SSkkeeff; ssKkEeff* to *ssKKEEff*) is found to vary complexly with the value in the heterozygote.

7. But if the pigmentation frequencies are subjected to an inverse probability transformation (WRIGHT 1920) the values in heterozygote and homozygote are found to have an approximately linear relation. The transformation does not bring about an approach to constant difference between *Sskkeeff* and *SSkkeeff*, although it does for *ssKkEeff* and *ssKKEEff*, which is taken to be a second indication of a difference in nature of primary effects between the (*KEF*) and *S* genes.

8. Following WRIGHT (1920) the frequency fields are considered in terms of some hypothetical material whose concentration P at a particular point of the skin must equal or exceed a minimal value P_0 for the ultimate formation of pigment. Most aspects of the observed regional and genotypic variation of pigmentation frequency can be accounted for if it is assumed that:

(a) the P of a particular skin point varies somewhat from animal to animal of the same genotype;

(b) the average value either of P, or of P_0 or of both, in the presence of a given gene combination, varies systematically from point to point over the skin surface;

(c) *K*, *E*, *F* have a constant additive effect on either P or P_0 throughout the anterior and posterior halves of the body, the anterior increment being nearly three times as large as the posterior;

(d) *S* has some sort of multiplicative effect on one of the two variables, P or P_0.

9. As reported by ONSLOW (1915) but somewhat doubted from the results of subsequent workers, tyrosinase can generally be detected in extracts of skin which is forming pigmented hairs; but it is either absent, or greatly diminished, in extracts of those skin regions of spotted mice which are forming unpigmented hairs.

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BAR-EYED MOSAICS IN DROSOPHILA MELANOGASTER

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Received June 7, 1938

FOR investigating certain aspects of the general problem of gene action it would be a great help if it were possible to substitute a gene for one of its alleles at the 2-cell stage, at the 4-cell stage, and so on, successively, at known later stages during development. The mere statement of such a hopeless ideal makes it at once clear that by such a method we could obtain information on the time during development when a gene is effective in producing any of its known effects, on the duration of its action, and on the rate at which it acts at every stage of any process in the developmental nexus which it affects.

Of the methods available at present for the study of gene action there are at least two which make an approach to this general method of gene substitution during development; namely, transplantation, in those cases where the implant and the host differ in some one or a few genes, as in the studies of BEADLE and EPHRUSSI (1937), and secondly, the use of mosaics, which are in effect spontaneous orthotopic transplants that avoid the distorting effects of an operation.

Of these methods each has its distinct advantages and drawbacks. The method of transplantation is especially suitable for studying the transport of materials from the organism to a part and in detecting the contribution of the part to the rest of the organism, and so gives promise of the eventual isolation and chemical identification of the diffusible substances involved. While the time relations can be known by this method, the results have not as yet led to a knowledge of the time course of any reaction in quantitative terms. On the other hand the method of analysis by the use of mosaics does supply quantitative data in suitable material, but the time relations, at least for spontaneously produced mosaics, are still not precisely known. An account of the results obtained by these two methods with full reference to the literature is included by GOLDSCHMIDT (1938) in his recent comprehensive exposition of the facts and their implications, relating to the whole subject of physiological genetics.

The present paper is concerned with white-eyed mosaics in the bar series of Drosophila. Previous reports on the mosaic condition of the eye of *Drosophila melanogaster* are those of BRIDGES (1925), STURTEVANT (1927) and HERSH (1934) in which the mosaic condition was produced by the use of Minute-n. PATTERSON (1929) who produced eye mosaics by x-raying larvae at different ages showed that the facet-producing reaction in the

wild type eye extended practically throughout the entire egg-larval period, a result which has been confirmed by MARGOLIS and ROBERTSON (1937) in their determination of the temperature effective period for the wild type eye. HASKINS (1935), whose main interest was in the determination of the genic volume at the white locus, obtained mosaics by x-raying larvae at age 18 to 24 hours and concluded that at that age there are about 20 cells in the eye anlage.

The data on white bar-eyed mosaics presented below were obtained by the use of Minute-n. Mosaics from matings of *fBMn/fB* ♀ ♀ × *w* ♂ ♂ were recovered at 20°, 25° and 30°C, and in addition mosaics from *fBMn/fB* ♀ ♀ × *wBB* ♂ ♂ matings were obtained at 25°C. In these experiments the flies were otherwise roughly isogenic, but not strictly so, since there was no prolonged period of suitable crossing and inbreeding of the stocks previous to doing the experiments.

The fluctuations from the temperatures mentioned above were less than a degree in either direction. The usual variation of the incubators was about 0.7° either way, except that in the experiments involving ultrabar at 25°, the temperature rose to nearly 27° for a period of about 24 hours.

The data on nearly 20,000 flies are summarized in table 1, where it can be seen that the number of mosaics among the *Mn* females is about 1 percent or less; more precisely 0.97 at 20°, 1.15 at 25° and 0.42 at 30° in the mosaics among the *fBMn/w* females. Among the *fBMn/wBB* females raised at 25° there were found 18 mosaics in a total of 1728 flies, or 1.04 percent. But this may be misleading, since in this series 525 *Mn* females were recovered before the first mosaic was found; 1310 *Mn* females were examined before the fifth mosaic was in hand. Four of the total 18 *Mn* mosaics in this series were found among the progeny of the same female which incidentally also produced two gynandromorphs (not included in table 1). Taking these details into account, we might estimate that the percentage rate of occurrence of *Mn* mosaics in this series which it is fair to compare with the results of the other series is perhaps more nearly about 0.5 percent rather than the 1.04 percent given directly by the data. Furthermore, it was in this series that the temperature rose to 27° for about 24 hours.

The non-*Mn* mosaics that appeared among the *fB/w* flies comprised 0.18 percent of the non-*Mn* females at 20°, and 0.32 percent at 30°. Unfortunately, no record was made of the non-*Mn* mosaics among the flies of similar genetic constitution raised at 25°, since at the time these flies were raised, the elimination of the *Mn* chromosome was accepted as the mechanism of mosaic formation instead of somatic crossing over which STERN (1936) has shown to be a much more likely explanation. Non-*Mn* mosaics among the *fB/wBB* flies raised at 25° comprised only 0.08 percent

of the total; 2 out of 2626 (table 1). Consequently, the presence of *Mn* increased the frequency of mosaics (somatic crossing over) from 4 to 10 times over that found in the flies of similar constitution but which lacked the *Mn* factor.

The data allow no very certain conclusion to be drawn in regard to the effect of temperature on somatic crossing over, although there seems to be a distinct tendency for the rate to be lower at 30° than at 20° or 25°. PLOUGH (1921) found that 31.5° caused little or no change on gametic crossing over in any part of the X chromosome. STERN and RENTSCHLER (1936) found in experiments involving *Mn* a lower rate of somatic crossing over at 30° than at 17° and 25°C.

The data on facet number of the mosaic-eyed flies are given in table 2. Before discussing the quantitative aspects of the data it might be men-

TABLE 1

Summary of the data. The first three columns of data give the numbers of offspring from matings of fBMn/fB ♀♀×w♂♂; the last is for the cross fBMn/fB ♀♀×wBB ♂♂.

	20°	25°	30°	25°
Minutes	2155	1286	1423	1710
Non-Minutes	2835	1730	1894	2624
Mn mosaics	21	15	6	18
Non-*Mn* mosaics	5	—	6	2
fB males	2373	1462	861	2301

tioned that mosaic spots of forked bristles and abnormally pigmented abdomens were noticed from time to time but no systematic study was made. In respect to the eyes no flies were found in which both eyes showed the mosaic condition. The right and left eyes were equally affected. In the mosaic eye the white patch was a larger or smaller segment that extended to the margin of the faceted area. There was not a single specimen in which the white area was entirely surrounded by red facets. STURTEVANT (1927, figure 4), however, has found that such a condition does occur. The larger the patch of white facets, the greater seems to be the distortion in shape of the faceted area over what is typical for bar, variable as bar eye itself is. Although distorted, the larger mosaic eyes tend to approach the wild type in outline. In two notable specimens (table 2) in which the "mosaic" eye was entirely white but somewhat smaller than wild type, the eye was actually oval in outline. This raises a point in regard to the qualitative effect in the bar series, namely, the indentation on the anterior margin of the faceted area. The conclusion seems obvious that the developmental antecedents of this indentation are not produced in the early stages of development.

With but two exceptions, found among the *fBMn/wBB* females, the

mosaic eye was always a single unified area. In one of these there was a group of 5 white facets on the ventral border, separated about 1 mm from the rest of the faceted area. The other exception was red-and-white banded. Beginning with the dorsal border of the eye and proceeding ventrally this specimen showed the following order of red and white facets: 1 white, 8 red, 8 white, 14 red, 3 white and 2 additional red facets along the edge of

TABLE 2

Facet number for the mosaics listed in table 1. The first three columns are for the mosaics from matings of fBMn/fB ♀ ♀ ×w♂♂, the last is for those from the cross fBMn/fB ♀ ♀ ×wBB ♂♂. For each mosaic there is given first the total facet number in the mosaic eye, followed in parentheses by the number of white facets, which in turn is followed by the total facet number in the opposite eye. For example, 203 (1) 189 indicates that in the mosaic eye were 203 facets 1 of which was white; in the opposite eye there were 189 red facets. In the last two columns the data below the cross-bars are for non-Mn mosaics.

20°			25°			30°			25°		
203	(1)	189	135	(4)	126	93	(14)	87	25	(11)	26
238.5	(7)	199.5	137	(9)	118	133	(41)	78	28	(1)	28
241	(8)	197.5	138	(8)	104	300	(143)	190	28	(2)	32
247.5	(19.5)	210	138	(12)	111	310	(7)	185	28	(13)	34
249	(39)	312	149	(2)	—	537	(537)	103	31	(2)	32
264	(24.5)	263.5	149	(3)	140	602	(455)	117	31	(23)	31
267	(9)	277.5	156	(21)	110				32	(3)	33
275.5	(18)	314.5	164	(4)	152	92	(3)	108	34	(9)	27
300	(6)	296.5	172	(2)	142	111	(4)	78	36	(9)	36
302	(64.5)	208.5	181	(6)	146	131	(8)	128	36	(12)	33
314	(105.5)	215	197	(55)	131	132	(29)	105	36	(31)	35
325	(60)	276	206	(24)	170	216	(2)	108	37	(6)	32
335	(11.5)	323	267	(60)	179	544	(62)	219	37	(11)	30
339	(7)	303	286	(84)	135				39	(8)	—
398.5	(146.5)	312	306	(113)	156				40	(39)	35
403	(109)	270.5							41	(13)	36
410.5	(106)	295.5							42	(16)	38
448	(20.5)	419							45	(12)	42
483.5	(263.5)	338									
500	(500)	300							36	(17)	37
619	(393)	197.5							44	(8)	34

the band of 8 white facets, giving a total of 12 white and 24 red facets in the mosaic eye. The opposite eye of this specimen possessed 33 red facets.

STURTEVANT (1927) reported that in the mosaics found by him among *BBMn/w* females, the number of red facets in the mosaic eye is greater than that found in the normal eye of the same fly. This is the case also for the mosaics found by us among the *fBMn/w* females raised at 25°. The difference in number of red facets between the mosaic and the non-mosaic eye ranges from 5 to 67, with an average of 22 facets in favor of the mosaic eye. The same relationship was not found in the other three sets of mosaics

reported here (see table 2). For example, in the mosaics among the *fBMn/w* females raised at 20° sometimes the mosaic eye had a larger number of red facets, and sometimes the non-mosaic eye had the larger number of red facets. The average difference, about 10 facets, is in favor of the non-mosaic eye. The other two series of mosaics depart still more widely in this respect. It seems clear that the greater number of red facets in the mosaic eye compared to the non-mosaic eye of the opposite side, commonly found in the *BMn/w* and *BBMn/w* mosaics at 25°, is merely a special case of some more general relationship. To give much importance to the larger number of red facets in the mosaic eye at 25° would be perhaps to regard standard conditions at 25° as a base of reference favored by nature for revealing the fundamental principles of genic action. What is obviously needed is a more comprehensive view based on a study of the quantitative relations exhibited by adequate data. But it is, nevertheless, clear that when the mosaic eye has a larger number of red facets than the opposite eye, then facets are formed in bar tissue that would not have had them otherwise (STURTEVANT 1927).

The facet counts of the individual mosaics (table 2) show, in spite of the variability, that with increasing size of the mosaic eye, the number of white facets increases, but it is also clear that the increase cannot be regarded as due simply to the white facets. As pointed out above, sometimes the number of red facets in the mosaic eye is greater than in the opposite eye and sometimes not.

In the attempt to find some simple uniformity in the data, it was discovered that when the number of white facets (y) is plotted logarithmically against the total number of facets (x) in the mosaic eye, the data for each series fall within a straight band which conforms to the simple power function

$$y = bx^k$$

known to biologists as the relative growth function or the equation of allometry, but it will perhaps do no harm to point out that power functions are among the most common relations found in nature.

The slope of the band which gives the value of k is about 5 in each case, but the band is shifted toward the left as the order of magnitude of eye size for any set of mosaics decreases. In other words, the value of b increases in flies of genetic constitution for smaller eye size and for those raised at higher temperatures, which in the flies under discussion also reduce the eye size.

For figure 1 the data for the two sets of mosaics raised at 25° and the one raised at 20° given in table 2 were seriated in suitable size classes on the basis of total facet number in the mosaic eye. This procedure gave the

points plotted on the graph, so that each point in the figure represents usually the average of from 3 to 6 mosaics. However, several of the points, especially those for large eye size, represent single specimens. The fitted lines of the figure were calculated by the method of averages, and are ex-

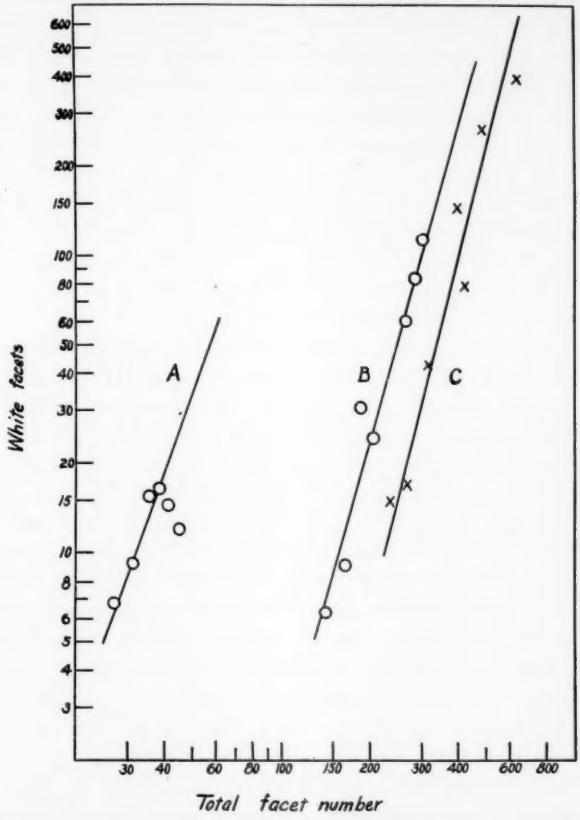

FIGURE 1.—The number of white facets (y) plotted logarithmically against total facet number (x) of the mosaic eyes; A, *fBMn/wBB* mosaics at 25°; B, *fBMn/w* mosaics at 25°; C, *fBMn/w* mosaics at 20°.

tended upward to the point where y = x, about which we shall have more to say below. (The point, x, 45; y, 12, which departs most widely from line A was not included in the calculations.) That the data conform to the power function implies that the increase in total facet number in the mosaic eye over the opposite eye is not due alone to the white facets. For certain portions of the curves conformity to the power function requires

a larger number of red facets in the mosaic eye than in the opposite eye and sometimes a smaller number. If the increase were due alone to the white facets it is clear that the data would not conform to the power function.

The calculated values of k are lower than the slopes determined graphically from the bands given by the plotted points for the individual specimens. The k value for the *fBMn/wBB* mosaics (fig. 1, A) is about 2.81. The value of k for the *fBMn/w* mosaics at 25° is 3.53 (fig. 1, B) and 3.86 at 20° (fig. 1, C). The values of log b for curves A, B, C are, respectively, −3.238, −6.744 and −8.044. These values can be considered only as approximately accurate.

Although the values of k differ it will be seen from figure 1 that the curves are roughly parallel to one another. This is due to the circumstance that the angles which the curves make with the x-axis are all in the neighborhood of 75°, a region where the tangent begins to show a relatively large change for a small increase in the angle.

The smaller the order of eye size in any set of mosaics, the greater is the shift of the curve toward the left. That is, the larger the value of b then the greater the shift of the curve to the left. It is clear from the figure that the main difference between these three sets of *Mn* mosaics is a difference in the constant b of the power function, but the value of k for the *fBMn/wBB* mosaics at 25° is lower than the k-values for the *fBMn/w* mosaics at 20° and 25° which are closely similar to each other.

On the purely mathematical side k is the ratio of the percentage increase in y to the percentage increase in x. If we take the value of k as about 4, then in biological terms the number of white facets increases about 4 times over that of the total eye. Consequently we soon come to a value of the function when y = x, that is, at this point, the eye will be entirely white. It can readily be shown that this point of the curve is given by the relation

$$\log y = (\log b/1-k).$$

The value when y = x for the *fBMn/w* mosaics (fig. 1, B and C) is 464 facets at 25° and 656 facets at 20°. These values are of course approximately average values. If we estimate the range of variability from the width of the bands obtained by plotting the mosaics individually, then the value of the function when y = x is from about 375 to 525 at 25° and from about 500 to 700 at 20°. In the set of *fBMn/w* mosaics at 25° none was found in which the eye was entirely white, but in the 20° series a remarkable specimen was found in which the "mosaic eye" was entirely white, having 500 facets; the opposite eye had 300 red facets.

For further discussion of the data in biological terms, it is necessary to make an assumption in regard to the relation between flies at different

points of the curve. The most direct and perhaps simplest assumption in this respect is that the greater the number of white facets then the earlier the event (somatic crossing over) occurs which changes the genetic constitution of certain cells and their descendants in the optic disk. This assumption is plausible on general grounds and fortunately it also has direct experimental evidence from full eye to support it. PATTERSON (1929) found that the younger the larvae were when treated with x-rays the larger on the average was the white-faceted area. In his experiment the change was only in genes for eye color.

In the *Mn* mosaics the change in genetic constitution for eye color denotes also a change at the same time in genetic factors for eye size at the bar locus. If somatic crossing over occurs to the left of *BMn* in *fBMn/w* females then there is no change in the genetic constitution at the bar locus although some cells will have the constitution for white facets *wfBMn/w* in an optic disk which otherwise is *fBMn/w* in constitution, and so will produce red facets. For a change in the genetic constitution at the bar locus the somatic crossing over must be to the right of *Mn* (we may neglect double crossing over). Fortunately, for this aspect of the problem STERN and RENTSCHLER (1936) worked with characters which enabled them to conclude that 118 out of 120 cases were derived from crossing over to the right of *Mn*. In the light of their evidence we may regard the mosaic condition of the eye to be, in practically all cases, a consequence of crossing over to the right of *Mn*.

When crossing over occurs to the right of *Mn* in *fBMn/w* females, the resulting cells have the constitution *fBMn/fBMn* and *w/w*. The former cells presumably die from the lethal effect of homozygous *Mn*, leaving the cells for white facets and wild type eye size in an optic disk which is otherwise *fBMn/w* in constitution. The earlier this event occurs, the larger on the average will be the white spot in an eye which is itself larger than it otherwise would have been as shown by the non-mosaic eye of the opposite side. The value when $y = x$, 464 facets for the *Mn* mosaics at 25° and 656 facets for those at 20°, gives the average size expected when the crossing over takes place at the latest possible time in development for producing an all white eye in *fBMn/w* females. If the event happens earlier the eye will, of course, be all white and be still larger and more nearly approach the size of the wild type, about 750 facets at 25°, about 850 to 950 facets at 20°. The main conclusion from these considerations is that the action of the bar factor up to the latest time for the occurrence of an all white eye is such as to depress the size of the eye by about 285 facets at 25° and by about 200 to 300 facets at 20°. But, furthermore, it may be considered that this latest possible time for the production of an all white eye which is smaller than the wild type by nearly 300 facets may be regarded as the

time of appearance of the first cell in the optic disk in heterozygous bar females, which in this case also contains *Mn*. This of course does not necessarily mean that in the wild type at the same corresponding time of development there are a sufficient number of cells already present in the optic disk to differentiate later into about 300 facets. It may be taken to mean only that the difference at this time between wild type and heterozygous bar, projected forward as it were and stated in terms of completed development, has a value of about 300 facets.

What the actual processes are which bring about the difference remain of course to be discovered, but the most obvious suggestion would concern itself with localized differences in cell divisions in the optic disk, or in the cells whose descendants later form the optic disk. These may be differences in the time or date of the occurrence of mitotic cycles in this part of the body of the fly, as well as differences in their duration and in the rate at which they proceed. Such differences would necessarily need to be referred back to prior differences in the cellular physiological processes between the wild type and the members of the bar series. While the differences in the cellular physiological processes at the beginning of development would constitute the first links in the chain or nexus of causes leading from the genes to their effects, they may well be the last in order of discovery.

The third set of *fBMn/w* mosaics may be disposed of more briefly. At 30° only 6 *Mn* mosaics and 6 non-*Mn* mosaics were found among the progeny of the *fBMn/fB* ♀ ♀ × *w* ♂ ♂ crosses. Although the number is small yet the range in size both of the entire eye and of the mosaic spot is considerable (table 2). Assuming a conformity of the meager data to the power function, a graphic determination of the slope gives about 1.76 for the *Mn* mosaics and about 1.70 for the non-*Mn* mosaics, but the curve for the *Mn* mosaics is considerably to the left of that for the non-*Mn* mosaics, that is, the *Mn* mosaics give the larger value of the constant b. In this series a specimen with an all white eye containing 537 facets was found among the *Mn* mosaics; the opposite eye possessed 103 red facets. Apparently the usual distortion of developmental processes which takes place at supranormal temperatures comes to expression also in these mosaics, as shown by the low value of k.

In these three sets of mosaics from *fBMn/w* at 20°, 25° and 30° the white spot will have the constitution *w/w* in an optic disk which is otherwise *fBMn/w* in constitution, if we accept somatic crossing over to the right of *Mn* as the mechanism of mosaic production. These three sets thus agree in having a change in constitution from heterozygous bar to homozygous wild type at the bar locus, in other words, a genetic change for a larger eye.

The other set of mosaics which we still have to discuss is the complement

of the others in the sense that in these flies there is a change to a genetic constitution for smaller eye than that with which development of the egg started. That is, after somatic crossing over to the right of *Mn* in *fBMn/wBB* females we have *wBB/wBB* cells in an optic disk otherwise *fBMn/wBB* in genetic constitution. Consequently in the *fBMn/wBB* flies as the change in genetic constitution happens earlier and earlier we should expect to get relatively a larger and larger number of white facets but the whole eye would be expected to become smaller and smaller. The eye would be expected to change in size from that found in heterozgyous bar ultrabar toward that found typically in homozygous ultrabar.

If there were no other condition acting to disturb in any way the simple operation of this process then a logarithmic plot of the number of white facets against total facet number in the mosaic eye should give a line of negative slope. However, when plotted the band of points for these mosaics has a positive slope (k is 5.2), while the value of k calculated from the grouped data, as mentioned above, is 2.81. Furthermore, the value is 61 facets at the point where $y = x$. But if, in this case, we expect on the basis of the mathematical relations to find a "mosaic" eye, all white containing 61 facets, we clearly draw an inference from the mathematical relations that falls beyond the limits allowed by biological fact. This follows, since flies of the constitution *fB/wBB* raised at 25° have a range in facet number from 36 to 47 with an average at 40.8 ± 0.37 facets; in *fBMn/wBB* flies at 25° the average is 36.0 ± 0.43 facets. If in the flies with the constitution *fBMn/wBB* there is a change in the optic disk during development to *wBB/wBB* the eye clearly would be expected to become smaller as the number of white facets increases, but the data in fact show that on the average the eye becomes larger. How is the apparent contradiction to be resolved? The explanation seems to be quite simple.

In the case of the *fBMn/w* mosaics the change in genetic constitution at the bar locus is one for a larger size of eye. If there were no variability in eye size, that is, if all flies of the given genetic constitution had the same size of eye, then the mosaic-eyed specimens among the *fBMn/w* females would be the only ones to differ in facet number. The mosaics would differ among themselves depending upon whether the somatic crossing over occurred earlier or later. But, of course, in actual fact the flies of any given genetic constitution show the usual variability. The variability in the mosaic specimens is superimposed upon the ordinary variability. As the change in genetic constitution happens earlier and earlier a larger and larger eye results which in time extends beyond the upper range of the ordinary curve of variability for flies with the constitution *fBMn/w*.

But in flies with the constitution *fBMn/wBB* when the change in genetic factors occurs the result is a constitution for smaller eye size. As

the somatic crossing over happens earlier and earlier the eye would be expected to become smaller and smaller but have a relatively larger number of white facets. Consequently, if the mosaic-eyed flies among the *fBMn/wBB* flies were the only ones which differed in facet number, then the smaller-eyed mosaics would have the larger number of white facets, resulting in a negative value of k. But the process of mosaic formation is superimposed upon the processes which produce the ordinary variability, and in this case practically within the limits of the ordinary curve of variability. The consequence is first to broaden the band within which the mosaics fall when plotted individually, and this in turn merely reduces the value of k below that found in the *fBMn/w* mosaics,—from nearly 4 to 2.81. The width of the band can be readily determined from the extreme values given by the two mosaics 25(11) and 45(12). Furthermore, the specimen in this series with 40 facets, 39 of which are white, is an indication that the change in constitution may happen early enough to give practically an all white eye yet still the eye may be on the upper limit of the range in total facet number. In this series the value $y = x$ at 61 facets cannot be given biological meaning.

A different opinion might be advanced that the positive value of k in this case, where a negative value is expected, is a consequence of a growth stimulating influence of an intercellular nature that arises from the contact of tissues of different genetic constitution (see STURTEVANT 1927, also GOLDSCHMIDT 1938, p. 177). An examination of the *fBMn/wBB* mosaics (table 2, column 4) shows that in the mosaic eye the range is from 25 to 45 facets, while in the non-mosaic eye of the same flies the range is from 26 to 42 facets. The failure of the range downward to be reduced and the range upward to be perhaps slightly increased favors the view of an intercellular influence. But in any case the effect is quite small. It seems that the irregular fluctuating variability itself is sufficient to bring about a positive value of k in the *fBMn/wBB* mosaics.

The bar type of eye may be regarded as a form of localized dwarfism. Such marked defective departures on the minus side of the wild type or normal are usually called arrests in development, of which there are many instances but which need no extended discussion here. But it is clear that not all so-called developmental arrests are the end effects of the same essential type of defective process. We may distinguish two main types. There is first the type in which a normal anlage is produced with later a loss by atrophy or autolysis, as in the vestigial alleles (GOLDSCHMIDT 1935). In some cases the loss may be by an autotomy. The second main type of localized dwarfism would stand in contrast to the first type and be exemplified by any case in which there is no actual loss but in which the apparent loss represents material that as an anlage or as a portion of an

anlage was developmentally never produced at all, such as defective endosperm in maize (MANGELSDORF 1926; see especially fig. 59). This second main type of localized dwarfism is an agenesis, and likewise comprises at least two distinct kinds. There is first the type in which the process in the mutant defect shows the same time course but stops abruptly at some point in the reaction which goes to asymptotic completion in the wild type or normal. This is the type of true arrest. In the second kind of agenesis the defective process goes to completion but has a lower entirely different asymptote than the normal, as in the case of defective endosperm mentioned above.

The data on bar-eyed mosaics clearly do not favor the view that in bar an anlage for a full eye is produced and then partially destroyed by autolysis to give bar eye, but rather that in bar the materials are never produced. The localized dwarfism of bar eye is not a loss by some form of atrophy or autolysis as in vestigial but only an apparent loss brought about by agenesis, as the following argument is intended to show.

If in *BMn/w* females an anlage for full eye is produced it will necessarily, on the basis of the given genetic constitution, be also one for the production of red facets. Now if because of the presence of bar a part of the anlage is lost by autolysis the eye will be smaller. It will show the bar effect. In such females when somatic crossing over occurs, leaving a genetic constitution for full eye, less of the original anlage would be destroyed and so, on this view, the eye would be larger; and the earlier the change in genetic constitution, the larger still would be the resulting mosaic eye. But these mosaic eyes are not only larger in size, they also possess a larger white spot. So far as the white facets are concerned, we require a process of some sort which will give more and more white facets, and not merely a process which destroys less and less material for the production of red facets. A process which destroys less and less material for the production of red facets although leaving a larger total eye than that found in bar will not produce more and more white facets. Bluntly and briefly, more and more white facets cannot result from a process which merely destroys less and less material for red facets. If this general argument is justified then it is valid to draw the inference that in bar eye we are dealing with a localized dwarfism that is not an atrophy but an agenesis. There is an agenesis of the facets which are lacking in bar but which are present in the wild type. Furthermore, the analysis (see HERSH 1934b) of DRIVER'S data on ultrabar shows that, at least for the reaction during the temperature effective period, the agenesis is very likely not a true arrest as defined above but a process with a different asymptote than that found in the wild type (but see GOLDSCHMIDT 1938, p. 76 and p. 216).

What a part becomes, according to the well-known formula, is a function of its size and of its position in the whole at the time of the embryonic

determination of the part. The data on mosaics support the view that the bar factors act by altering the growth, presumably by reducing the number of cell dividisons in the optic disk. After the loss of the bar factor as the result of crossing over in a *fBMn/w* fly the cell with wild type constitution divides more rapidly because of the absence of bar, and after somatic crossing over in a *fBMn/wBB* fly the addition of a bar factor reduces somewhat further the number of cell divisions in the optic disk, but not enough to shift the curve of variability of the *fBMn/wBB* flies very far toward homozygous ultrabar.

SUMMARY

Data on white bar-eyed mosaics of Drosophila recovered at 20°, 25°, and 30° in *fBMn/w* flies and in *fBMn/wBB* flies at 25°, conform as an approximation to the power function, $y = bx^k$. (y is the number of white facets, x, the total facet number in the mosaic eye, b and k are constants.) The values of k for the 4 sets of mosaics in the order mentioned are 3.86, 3.53, 1.76 and 2.81. As the order of eye size in any set increases the value of the constant b decreases. The presence of Minute-n increases from 4 to 10 times the rate of mosaic formation over that found in the corresponding non-*Mn* flies of the same experiments. The data favor the view that bar eye considered as a localized dwarfism is the result of a process of agenesis and not one of atrophy or autolysis as in the vestigial alleles. Although the time relations are unknown the inference can be drawn from the mathematical relations that in *fBMn/w* mosaics there is a time during development which is the latest time for the change at the bar locus to wild type to result in an all-white eye. Up to this time the action of the bar factor is to depress the eye size by nearly 300 facets.

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THE INDUCTION OF DOMINANT AND RECESSIVE LETHALS BY RADIATION IN HABROBRACON[1]

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Received June 15, 1938

INTRODUCTION

GENETIC literature includes much discussion of lethal factors. Texts treat the subjects of sex-linked and autosomal lethals, lethals linked with visibles, balanced lethals and semilethals. There are many studies of induction of lethals by X-radiation and other physical agents including correlation of number of lethals with dosage given. Almost without exception the lethals referred to are recessive, transmissible from one generation to another through the heterozygote.

Dominant lethals, not transmissible since zygotes receiving them die, and evident only in their mass effect, have been discussed but little. In organisms with the ordinary type of reproduction dominant lethals would be difficult to distinguish from direct inactivation of germ cells although MULLER (1927) attributed the partial sterility of X-rayed males in Drosophila to dominant lethals, pointing out that although they could not be detected individually "their number was so great that through egg counts and effects on sex ratio evidence could be obtained of them *en masse*. It was found that their numbers are of the same order of magnitude as those of recessive lethals."

In the parasitic wasp Habrobracon, in contrast to such organisms as Drosophila, the occurrence of dominant lethals in the sperm can be readily distinguished from direct killing of the male gametes, as explained below. For this reason the experiments reviewed and reported in the present paper were undertaken and the results indicate that the induction of dominant lethals by radiations can scarcely be doubted.

For an understanding of the exceptional method of reproduction and heredity in this insect the following facts should be borne in mind.

Females are diploid and develop from fertilized eggs. Normal fertile males are haploid and develop parthenogenetically from unfertilized eggs.

[1] The work reported in this paper has been greatly aided by grants from the Committee on Effects of Radiation on Living Organisms (National Research Council). Most of the recent experiments have been carried out at the University of Pennsylvania, with the breeding work done at the Zoological Laboratory, the cold rooms for storage supplied by the Department of Anatomy at the Medical School and the X-ray treatments given by Dr. Raymond Zirkle at the Department of Radiology of the University Hospital. During the summers the breeding work has been done at the Marine Biological Laboratory, Woods Hole, Mass., and the X-ray treatments have been given there.

Biparental sterile (or near-sterile) males are diploid, developing, like females, from fertilized eggs. The haploid males have been called uniparental and azygous in contrast to the biparental zygous males and females.

Unmated females produce azygous sons only. Mated females also produce azygous sons from unfertilized eggs but in smaller numbers than unmated females and zygous biparental offspring from fertilized eggs. Whether the biparental offspring shall be daughters only or both sons and daughters depends upon the relationship of the male used in the mating. If the male is from a stock unrelated to the female, all the biparental offspring are daughters but if the male comes from a related stock, biparental sons as well as daughters are produced.

In general the azygous and biparental offspring from a mated female occur in the same ratio from the different vials through which the female is transferred (a, b, c, d, etc.) until her supply of sperm is exhausted. Subsequently (vials, e, f, etc.) azygous sons only appear.

Biparental males cannot conveniently be separated from their azygous brothers unless the mother has a recessive trait and the father has the allelic dominant. Orange-eyed females crossed with wild type (black-eyed) males produce black-eyed daughters and orange-eyed azygous sons. If biparental sons are produced they may be readily separated from their azygous brothers by their black eyes.

All daughters from outcrosses appear to be similar in viability to inbred females, although the latter are in general somewhat less fecund.

Fertilized eggs may be "female-producing" or "male-producting," the latter occurring only if parents are related. "Male-producing" fertilized eggs are less likely so hatch than "female-producing." Consequently there are more "bad eggs" and fewer biparental offspring if the mating has been with a related male, since the percentage of eggs fertilized is the same whether the male is related or unrelated. Biparental male larvae are also less likely to mature than female larvae. This further reduces offspring from related parents.

Two types of male sterility may be distinguished according as the eggs are fertilized or not. If the eggs are not fertilized, the mated female breeds like an unmated female, producing a large number of azygous sons. This occurs in the case of matings with biparental males which produce diploid sperm rarely capable of fertilizing the eggs. There are also males with abortive sperm ducts or testes which may readily be mated but transmit no sperm. Recent evidence indicates that sperm may to some extent be inactivated by high dosages of X-rays, so that they are unable to penetrate the eggs.

The most striking result of X-radiation of males is, however, the pro-

duction of sterility (or partial sterility) of a second type. In this case the sperm are not inactivated but are fully capable of fertilizing the eggs. Such fertilized eggs however, do not hatch because the sperm have a dominant lethal effect. Females mated to males with this type of sterility produce azygous sons only and in numbers equal to those produced by females mated to normal males; in other words, although they produce no females, due to dominant lethal effects of the sperm which have entered the eggs, they behave like mated rather than unmated females in respect to number of azygous sons. Habrobracon, then, is especially well suited for separating dominant lethal male sterility from sterility due to inactivated sperm on the basis simply of numbers of azygous sons produced by females mated to the males to be tested.

As to the nature of dominant lethals it is probable that they are due to extensive chromosomal alterations rather than to changes in restricted regions or in single genes. A lethal effect from a single gamete may be called dominant in contrast to a condition which must be present in both gametes and hence recessive.

Since in Habrobracon an unfertilized egg provided with a single set of genes develops normally (into a male) and since the addition of a second complete set by fertilization likewise results in normal development (into a female), it might be thought that the addition of a deficient set should not be lethal to the resulting zygote. In other words, if both 1n and 2n may develop normally, why should not $1n+(n-x)$ also develop normally? The explanation is doubtless to be found in genic balance. If $n-x$ is too small to act as a recessive, containing relatively extensive deletions for example, the balance should be so disturbed that development would be prevented. Theoretically the genic set in a sperm might become so extremely deleted by X-radiation that the fertilized egg would develop as an unfertilized egg into a male. No sex intergrades have ever been found in the treated material that might be interpreted as hyperploid males or hypoploid females. The sex types surviving after X-radiation have been fully as normal as in untreated stock.

Recessive lethal (or semilethal) factors may be a cause of "bad eggs." Half of the unfertilized eggs of a female heterozygous for a recessive lethal do not hatch.

Eggs may likewise be defective in their gross morphological or non-nuclear aspects because of unfavorable cultural conditions (very low humidity) or inadequate nutriment of females (feeding with honey instead of caterpillar juice) while females of certain genetic types lay withered eggs of irregular form.

Non-hatchability may then be due to non-nuclear causes, recessive lethals, dominant lethals or "male-producing" fertilization.

Table 1 is based on part on data published by C. H. BOSTIAN (1935) who counted eggs and offspring from females of an inbred orange-eyed stock which were bred unmated, or after mating to related or to unrelated males. Eggs averaged 21 per day regardless of the type of mating. Some of the unhatchable bad eggs from unmated mothers may be so because of recessive lethals in the stock and hence there is a somewhat greater viability of offspring (of which three-fourths are females) when mothers are mated to unrelated males. Matings with related males result in many unhatchable eggs. If the difference between bad eggs from matings of related and unrelated parents be added to the biparental sons from the

TABLE 1

Offspring per day of inbred orange-eyed females (stock 11-0) bred unmated or mated with wild type males. The average daily egg production per female is 21.

TYPE OF MATING	OFFSPRING PER DAY			
	AZYGOUS. ORANGE MALES	BIPARENTAL.		BAD EGGS
		WILD TYPE FEMALES	WILD TYPE MALES	
Female unmated (or mated to male unable to transmit sperm)	14.5	—	—	6.5
Outcrossing; male of an unrelated stock	4	12	—	5
Inbreeding; male of a closely related stock	5	5.5	1.7	8.8
Dominant lethals in all sperm	5	—	—	16

former, it appears that female-producing and male-producing combinations occur in equal numbers (5.5 of each type per day). The table also gives the expectation in case of a mating with a male having dominant lethals in all sperm. Here all fertilized eggs are unhatchable and offspring are limited to five azygous sons per day.

RECESSIVE LETHALS

In a discussion of azygotic segregations from heterozygous mothers (WHITING and BENKERT 1934) deviations from equality of mutant types and wild type were explained by linkage with recessive lethals, especially when the types were otherwise of normal viability. These deviations may be very great or relatively slight indicating that the lethal may be either closely or loosely linked with the mutant locus. Not infrequently also the pedigree shows that a certain female is heterozygous for a lethal since about one-half of her daughters are lethal bearing.

WHITING (1929), in a general account of X-radiation results with Habrobracon, presented evidence that certain daughters of treated parents

were carrying recessive lethals. Very few offspring (males) were produced if such lethal-bearing females were unmated, but if mated they were able to produce many daughters. When the number of their sons was extremely reduced, it was suggested that the lethals were either very numerous or were balanced.

MAXWELL (1935) found no reduction in fecundity of F_1 females and no deviations from the 1:1:1:1 ratio of F_2 males when the wild type P_1 females were treated with X-ray dosages of 2,500 r and subsequently mated to untreated cantaloup gynoid males. In a second experiment treatment of the cantaloup gynoid males with the same dosage before mating with untreated wild-type females was however very effective, reducing the average number of sons per day per F_1 mother from 7.06 to 4.59. Males subjected to higher dosages showed still greater reduction in these averages. The 21 control F_1 females each averaged six or more sons per day, with but two exceptions, while, among the daughters of treated fathers, 31 averaged six or more while 64 averaged three or less. This bimodality indicates that about two-thirds of the daughters of treated males carried at least one lethal. Among the 105 F_2 fraternities from treated males there were significant deviations from equality for cantaloup in four and for gynoid in four. MAXWELL's data suggest that recessive lethals are more readily induced in the sperm than in the eggs.

DOMINANT LETHALS INDUCED BY TREATMENT OF MALES

MULLER (1927) attributed the partial sterility of X-rayed males in Drosophila to dominant lethals. WHITING (1929) showed that in Habrobracon males were active and apparently uninjured after a dosage that was seven times as great as was required to induce complete sterility. With treatments below this sterilizing dosage, there was a decrease in percentage of daughters among the progeny with increasing dosages when the males were mated immediately after treatment, but there was a partial recovery of fertility as indicated by increasing percentage of females from subsequent matings on successive days.

STANCATI (1932) demonstrated dominant lethals in sperm of wild type males after treatment (2,500 r). The males were mated to females of a related stock with orange eyes, so that biparental sons were produced and could be detected. STANCATI reported numbers of offspring per vial and percentages of biparentals, including the biparental sons with the daughters. Not all matings were observed but the controls included "practically no unisexual fraternities." Among the treated, however, there were many unisexual fraternities, indicating sterilization of the males. From his figures it may be calculated that there were among the controls, 3.39 azygous sons per vial. This was increased to 4.66 among the treated

bisexual and to 4.94 among the treated unisexual fraternities. This increase might suggest inactivation of sperm, but this seems unlikely in view of later results and the low dosage. Some fraternities from unmated females may have been included among the unisexual treated. The biparental offspring, sons and daughters, are 6.25 per vial from the controls and 1.50 from the treated bisexual. This decrease is not compensated by the increase in azygous sons and hence the partial sterility of the fathers was in part at least due to dominant lethals.

WHITING (1936) showed that dominant lethals were induced in the sperm by neutrons. Treated males were mated to untreated unrelated females. While daughters decreased steadily with increasing dosages, there was no compensating increase in number of sons. Thus the sperm were not inactivated by the neutrons but were able to penetrate the eggs and prevent their development.

WHITING (1937 a and b) likewise showed a progressive increase in partial sterility due to dominant lethals when X-rayed males were mated to unrelated females. Table 2 (reprinted from WHITING 1937a) gives the data. Irregular fluctuation of sons per day may be noted. A slight increase in the 75,000 r group suggests possible sperm inactivation, but if this is significant it requires a far higher dosage than is necessary for complete sterilization by induction of at least one dominant lethal in every sperm.

TABLE 2

Offspring produced in a given number of days by untreated orange-eyed females (stock 11-o) mated to wild type unrelated males, controls and X-rayed with various dosages.
(From "The Collecting Net," Woods Hole, August 7, 1937.)

TREATMENT OF MALES	TOTAL EGG-LAYING DAYS	OFFSPRING		o ♂♂ PER DAY	+ ♀♀ PER DAY	SURVIVAL RATE
		o ♂♂	+ ♀♀			
Controls	379	459	1310	1.21	3.30	1.00
2,500 r	253	337	562	1.33	2.22	.67
5,000 r	309	419	191	1.36	.62	.19
7,500 r	361	627	57	1.74	.16	.05
10,000 r	72	62	5	.86	.07	.02
20,000 r	99	95	0	.96	0	
40,000 r	117	157	0	1.34	0	
75,000 r	515	890	0	1.73	0	
Unmated ♀♀	675	3191	0	4.73	0	

BISHOP (1937) showed by counting eggs and recording progeny that X-rayed males had fewer daughters than untreated and that there was a corresponding increase in bad eggs. Matings were between both unrelated and related parents. In the latter case biparental sons occurred but were not numerous enough to indicate decrease due to treatment.

WHITING (1938) showed that there was progressive decrease in biparentals, both sons and daughters, with increasing X-ray dosages when the treated males (wild type) were crossed with related females (orange-eyed). There was no compensating increase in number of azygous sons but this remained low as among the offspring of the mated controls.

For the four groups: controls, 2,500 r treatment, 5,000 r and 7,500 r, the daughters per day were respectively 1.83, 0.97, 0.31 and 0.06 and the biparental sons per day were 1.20, 0.59, 0.17 and 0.05. Fraternities from crosses of these stocks give a very high ratio of biparental sons, but nevertheless they fail to equal the daughters. It is of interest to note that decrease in daughters and in biparental sons is at the same rate, a fact that is taken to indicate that the radiation is no more lethal to one than to the other sex among the biparentals and that the type of fertilization (male-producing vs. female-producing) is not modified.

MAXWELL (1938) mated the same males on successive days to unrelated females. Dosages of 41,000 r and 142,000+r were administered to the two groups of treated males so that no daughters were produced by the treated. Sons per day averaged 1.05 for the controls, 1.41 for the lower treatment and 2.01 for the higher. Maxwell concludes that "Inactivation of sperm following higher dosages rather than partial exhaustion of sperm supply due to partial inhibition of spermatogenesis is indicated by the fact that there is no increase in males per day from the later matings."

DOMINANT LETHALS INDUCED BY TREATMENT OF FEMALES

Treatment of females by X-radiation was made by WHITING (1929). Mature eggs were shown to be less susceptible, while earlier stages were readily affected. Thus females fed on caterpillars and in actively laying condition had a postponed sterility since they produced offspring immediately after treatment (in vials *a*), but proved sterile thereafter. Honey-fed females, having no mature eggs, produced no offspring after equivalent dosages. In certain cases the postponed sterility was temporary for a few offspring might be produced in later life. Inviable pupae and larvae were sometimes observed after recovery, but data are not sufficient to prove that such were more numerous than usual.

In an experiment with low intensity but prolonged treatment of mated females (WHITING 1929), the percentage of daughters was decreased with increasing dosages, being 24.0−, 16.9− and 11.7− in the bisexual fraternities. No untreated controls were run. NEITA C. BOSTIAN (1931) X-rayed (3,200 to 6,400 r) inbred females mated to males of the same stock (no. 1). Offspring per female and percentage of daughters (vials *a* only) decreased with increasing dosages. When unmated females were treated (3,267 r) and subsequently mated to untreated males, offspring

per female and percentage of daughters were both higher than among the mated females treated with the lowest dosage. WHITING (1935), reporting experiments of JANE MAXWELL, ANNA R. WHITING and KATHRYN G. SPEICHER, showed that among the offspring of mated females X-rayed with dosages ranging from 3,500 to 7,000 r, the percentage of daughters decreased (38% to 7%) with increasing dosages. GREB (1933) showed that among bisexual fraternities from X-rayed mated females the average number of sons per mother was not significantly affected by a light dosage (2,000 r), although the daughters were much decreased. These experiments

TABLE 3. EXPERIMENT 11/3/37/TST

Offspring produced in a given number of days by orange-eyed females (stock 11-o), (controls and X-rayed with various dosages), which were bred unmated or were mated to unrelated untreated wild type males (stock 32) either before or after treatment.

MOTHERS	TREATMENT	DAYS FERTILE	TOTAL DAYS	o ♂♂	+ ♀♀	♂♂ PER DAY	SURVIVAL RATE	♀♀ PER DAY	SURVIVAL RATE	% ♀♀
Unmated	Controls	143	143	488		3.41+	1.00			
	1,500 r	81	81	225		2.78−	.81+			
	3,000 r	110	110	200		1.82−	.53+			
	6,000 r	20	67	28		.42−	.12+			
	12,000 r	20	103	22		.21+	.06+			
Mated	Controls	289	289	252	766	.87+	1.00	2.65+	1.00	75.25−
Mated	1,500 r	102	102	126	245	1.24−	1.42−	2.40+	.91−	66.04−
before	3,000 r	96	96	54	50	.56+	.65−	.52+	.20−	48.08−
treatment	6,000 r	12	85	13	5	.15+	.18−	.06+	.02+	27.78−
	12,000 r	20	100	4		.04	.05−			
Mated	1,500 r	86	86	49	112	.57−	.65+	1.30+	.49+	69.56+
after	3,000 r	139	139	70	250	.50+	.58−	1.80−	.68−	78.13−
treatment	6,000 r	16	70	3	15	.04+	.05−	.21+	.08+	83.33+
	12,000 r	20	106	3	20	.03−	.03+	.19−	.07+	86.96

suggest that dominant lethals are more readily induced in the sperm than recessive lethals in the eggs.

Two experiments have recently been completed in which females were X-rayed. I am indebted to DR. RAYMOND ZIRKLE and to the Department of Radiology, University of Pennsylvania Hospital, for cooperation in giving the X-ray treatments.

Females of an orange-eyed stock (11-o) were fed on caterpillars so that they were in active egg-laying condition. They were bred unmated or were mated to azygous males (collected from unmated mothers to avoid any chance of using biparental sterile males) of an unrelated wild type stock (no. 32). Thus, no biparental sons should be produced and percentage of

daughters should be relatively high. Females were subjected to X-radiation and matings were made either immediately before or immediately after treatment. Except for the controls, eggs were therefore treated in all cases, but sperm were X-rayed only when matings preceded treatments. Data are summarized separately (tables 3 and 4) since cultural conditions were much better in the second experiment resulting in larger numbers.

TABLE 4. EXPERIMENT 2/38/GS

Offspring produced in a given number of days by orange-eyed females (stock 11-o), (controls and X-rayed with various dosages), which were bred unmated or were mated to unrelated untreated wild type males (stock 32) either before or after treatment.

MOTHERS	TREATMENT	DAYS FERTILE	TOTAL DAYS	o ♂♂	+ ♀♀	♂♂ PER DAY	SURVIVAL RATE	♀♀ PER DAY	SURVIVAL RATE	% ♀♀
Unmated	Controls	225	225	1291		5.74−	1.00			
	1,500 r	86	86	382		4.44+	.90+			
	3,000 r	36	36	72		2.00	.41−			
	4,000 r	151	151	191		1.26+	.26−			
	5,000 r	88	88	109		1.24−	.25+			
	6,000 r	52	118	51		.43+	.09−			
	12,000 r	20	135	28		.21−	.04+			
	18,000 r	16	57	4		.07+	.01+			
Mated	Controls	209	209	292	953	1.40−	1.00	4.56−	1.00	76.55−
Mated	1,500 r	114	114	152	399	1.33+	.95+	3.50	.77+	72.05+
before	3,000 r	124	124	154	345	1.24+	.89−	2.78+	.61−	69.14−
treatment	4,000 r	123	123	55	70	.45−	.32+	.57−	.12+	56.00
	5,000 r	208	208	83	77	.40+	.29−	.37+	.08+	48.13−
	6,000 r	44	80	35	10	.44−	.37+	.13−	.03−	22.22+
	12,000 r	44	172	3	1	.02−	.01+	.01−	.00+	25.00
	18,000 r	20	103	11		.11−	.08−			
Mated	1,500 r	127	127	122	524	.96+	.69−	4.13−	.90+	81.12−
after	3,000 r	119	119	161	510	1.35+	.97−	4.29−	.94−	76.01−
treatment	4,000 r	186	186	106	434	.57−	.41−	2.33+	.51+	80.37
	5,000 r	150	150	108	266	.72	.52−	1.77+	.39−	71.12+
	6,000 r	80	97	22	91	.23−	.16+	.94−	.21−	80.53+
	12,000 r	28	212	18	19	.09−	.06+	.09−	.02−	51.35+
	18,000 r	20	103	8	5	.08−	.06−	.05−	.01+	38.46+

If there is no inactivation of sperm resulting from the treatment, the survival rate of sons per day (sons per day for the various treated groups divided by sons per day for the controls) (column 8, tables 3 and 4) should be the same for equal dosages in both experiments and whether mothers are unmated or mated before or after treatment. For the unmated series in both experiments where numbers of azygous sons are relatively high the rates are roughly parallel, but there is wide fluctuation in the four

mated series. In this connection it should be recalled that estimates of errors based merely on the numbers of offspring may be too small. A recessive lethal or semilethal occurring in a very few mothers may have considerable influence on averages of azygous sons. This is but one of the factors that may cause wider fluctuations than might be expected from the numbers appearing in the tables. Nevertheless it may be noted that in both experiments the groups in which sperm were treated have in general a higher rate of production of azygous sons. That this indicates sperm inactivation is doubtful in view of MAXWELL'S (1938) findings, but further tests would be of interest.

The decrease in azygous sons (as also in daughters) with increasing dosages is very marked at 6,000 r. At about this point complete sterility usually appears after vials *a*. The drop in average offspring per day is then due to the fact that the mother may live for many days subsequently without producing offspring. This postponed sterility may cause much fluctuation in averages if the length of life subsequent to the brief fertile period be greatly extended even in a single female. The "days fertile" (from time set until no more offspring were produced) are therefore lower (tables 3 and 4, column 3) for the females treated with higher dosages. In several of the 6,000 r groups (especially in experiment 2/38/GS, table 4) there were offspring in vials *a* but none in *b* followed by a few and sometimes several in *c* or *d*. This postponed temporary sterility suggests greater susceptibility of young oöcytes than of oögonia. Mature eggs seem to be but little affected and even after 18,000 r a few offspring may be produced.

Decrease in daughters per day is much more striking if matings are made before the treatment so that sperm are irradiated. Practically all daughters are eliminated by 12,000 r treatment of the sperm in the mated females. This is in agreement with the results from a closely similar treatment of the males (10,000 r) (WHITING 1937, see table 2 in this paper).

A few daughters are produced after 18,000 r treatment of eggs fertilized by untreated sperm.

The percentage of daughters drops rapidly when eggs and sperm are both treated, but if eggs alone are treated it is doubtful if there is any significant effect. The total number of offspring is very much reduced with higher dosages so that the apparent drop in percentage of daughters after treatment with 12,000 r and 18,000 r before mating (table 3) may be only a fluctuation. It was expected that a slight increase in percentage of daughters might appear on account of recessive lethals induced in the eggs. Were it possible to cause recessive lethals in all eggs escaping dominant lethal effects, the percentage of daughters might be increased up to one hundred. Further work may indicate whether this can be done, or whether eggs may be rendered unfertilizable but still able to develop normally into

males. Dominant lethals induced in the eggs should have no effect on the sex ratio.

SUMMARY

1. Dominant lethals may be induced in the sperm of Habrobracon by X-radiation of the males. At 10,000 to 20,000 r units all sperm have at least one lethal. With very high dosages, 41,000 to 142,000 r, some sperm are directly inactivated while many still remain active and able to carry dominant lethals into the eggs. Reduction of biparental sons and of daughters takes place at the same rate.

2. Recessive lethal-bearing daughters of treated males were twice as numerous as non-lethal bearing. Linkage with segregating visible mutants is sometimes indicated.

3. Treatment of females causes reduction in offspring per day, due either to the induction of dominant lethals or to direct killing of eggs. No change in sex ratio would be caused by dominant lethals in the egg.

4. If females are treated and subsequently mated, there is no appreciable reduction in male ratio, indicating that few, if any recessive lethals are induced in the egg.

5. Treatment of mated females causes a radical lowering of female ratio indicating that more dominant lethals are induced in the sperm than recessive lethals in the egg.

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THE DEVELOPMENT OF TWO TAILLESS MUTANTS IN THE HOUSE MOUSE

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Received June 30, 1938

INTRODUCTION

THE events that take place in the development of the mammalian embryo have not been subjected to an extensive causal analysis so far. The reasons for this are to be found mainly in the lack of suitable methods. It is not possible yet to use transplantation, isolation or vital staining techniques on mammalian embryos as they have been used on amphibian embryos. In the course of time it probably will be possible to analyze the mammalian embryo by transplantation and isolation just as thoroughly as has been done with the amphibian. For the present, however, the experimenter is not able "to take an active part in the course of events that take place during the embryogeny of the mammalian embryo," nor "to alter the course of events at a chosen point in a chosen manner and draw conclusions on their relations from the resulting changes." (SPEMANN 1936.)

A mutation that causes a certain malformation as the result of a developmental disturbance carries out an "experiment" in the embryo by interfering with the normal development at a certain point. By studying the details of the disturbed development it may be possible to learn something about the results of the "experiment" carried out by the gene. However to discover anything about the nature of the action of the gene is a much harder task. It is necessary for this purpose to be able to trace back all the results of the action to certain original causes. While the experimental embryologist carries out a certain experiment and then studies its results, the developmental geneticist first has to study the course of the development (that is, the results of the developmental disturbance) and can then sometimes draw conclusions on the nature of the "experiment" carried out by the gene.

In amphibian embryology the experimental analysis has led to an understanding of the causal relations of the normal developmental events. It is possible that the study of hereditary developmental disturbances and their causes in the mammalian embryo may contribute to knowledge of the causal morphology of mammalian embryology.

This paper restricts itself to the description of the development of an

[1] This research was aided by a grant from the Josiah Macy Jr. Foundation.

hereditary malformation which consists in taillessness in adult mice. So far it has not been possible to associate this malformation with a specific original defect, although a possible mode of origin is suggested and further investigations are being made along this line.

The genetics of the two strains of mice in which taillessness is produced (strains A and 29) has been described in outline in two papers (CHESLEY and DUNN 1936; DUNN 1937).

Taillessness in these strains is due to the interaction of the dominant gene for Brachyury T and a recessive allele t^0 in the A line, t^1 in the 29 line. The tailless strains breed true because of a balanced lethal condition. The homozygotes TT die at the eleventh day after fertilization (CHESLEY 1935), the t^0t^0 homozygotes die shortly after implantation, and the t^1t^1 homozygotes either before or just at implantation. Only the heterozygotes Tt^0 and Tt^1 survive to become tailless adults.

CHESLEY (1933, 1935) described the development of the short-tailed mouse, the heterozygote $T+$, and that of the lethal homozygote TT. It is proposed in this paper to describe the development of the tailless mouse in the A and 29 strains.

I wish to express my thanks to DR. L. C. DUNN for the suggestion of the problem and his help and criticism during the course of the investigation.

MATERIAL AND METHODS

The material used for the investigation of the development of the tailless mouse was obtained from matings of tailless by tailless ($Tt^0 \times Tt^0$ or $Tt^1 \times Tt^1$) and of Brachy by tailless ($T+ \times Tt^0$ or $T+ \times Tt^1$) or Brachy by heterozygous normal tailed mice ($T+ \times +t^0$ or $T+ \times +t^1$). The embryos were timed by the vaginal plug method. Male and females were left together for approximately fifteen to twenty hours, after which time the female was examined for a vaginal plug. Thus the timing of the embryos was accurate within $\pm$ 10 hours. Since there is a high degree of variability in the chronological and physiological age of the embryos it was sufficient for the purposes of this investigation to time the embryos within $\pm$ 10 hours in order to obtain a complete series of tailless embryos.

The embryos were obtained from the mother in the following way. The mother was killed with ether and the uterus with the ovaries was taken out and immersed in a .75 percent saline solution. The number of embryos, their position in the uterus, and any distinct size difference observable among the embryonic capsules were recorded. Up to the ninth day of development some of the litters were dissected out of their capsules, closely examined and recorded and then preserved for imbedding and sectioning. Since in these early stages of development the embryos are easily injured when dissected out of their protecting membranes, some of

the litters were preserved and sectioned in their capsules. From the tenth day of development on the embryos were always dissected out of their capsules for recording and preservation.

All embryos were fixed in Bouin's fluid. They were imbedded in paraffin and serial sections were made at 6μ to 15μ, depending on the size of the embryo. The sections were stained in Delafield's Haematoxylin and counterstained in eosin.

For the purpose of studying the development of the tailless mouse in line 29, 21 litters from matings of tailless by tailless, Brachy by tailless and heterozygous normals by Brachy or tailless were dissected out. A total of 155 embryos was obtained. Fourteen litters yielding 100 embryos were ten days or older. At this age future taillessness can be diagnosed: of these 100 embryos 27 were tailless. In litters younger than ten days (7 with a total of 55 embryos) future taillessness could not be diagnosed. In the A line 23 litters consisting of 202 embryos were dissected out for the study of the development of the tailless mouse. Of these 18 could be diagnosed to be tailless by gross and histological examination. Thirty-three more embryos of the total were known to be genetically tailless because they came from matings of tailless by tailless. In 13 litters yielding 105 embryos, which were younger than 10 days and resulted from Brachy by tailless or Brachy by heterozygous normals, future normal and tailless mice could not be distinguished from each other. For embryos older than 10 days, normal litter mates were used as controls. Besides these, ten normal litters were dissected out, yielding a total of 75 normal embryos which were also used as controls. Development proceeds identically in the tailless embryos of the 29 line and in those of the A line. The following description was first made for the 29 line and was then proved to be true also for the A line.

DESCRIPTION OF THE ADULT TAILLESS MOUSE

The morphology of the adult tailless mouse has been thoroughly described by KOBOZIEFF (1935). By means of a great many radiographic pictures he demonstrated the high degree of variability in the malformations of the sacral and caudal regions of the spine of the tailless mice. The adult tailless mouse in some cases does not possess the slightest trace of a tail, but in most of the cases there is a short filament present, consisting of skin and connective tissue only and varying in length from 2 to 10 mm. Malformations in the spine consist of fusions of lumbar vertebrae with each other or with the sacrum, or of simultaneous fusions of both lumbar vertebrae inter se and with the sacrum, or of fusions of the entire lumbar region with the sacrum, and of fusions in the caudal region. Furthermore there is found a failure of ossification in the spine and incomplete fusion

in the middle line of the vertebrae. The number of vertebrae in the lumbar and sacral regions of the tailless mice is constant in the majority of cases, five in the lumbar and four in the sacral region. However a small degree of variability has been found in other cases, the number of vertebrae in the regions mentioned above being augmented or reduced. There is a high amount of variability in the manner of termination of the spine. It may terminate in the tailless mouse anywhere between the first sacral vertebra and the fourth caudal. Sometimes the spine ends quite abruptly as if it were cut off at the level of the intervertebral disc. Most often, however, the spine seems amputated not at the level of the intervertebral disc but across a vertebra. The last vertebra may be rounded off, it may lack all processes or it may be elongated more or less. In certain cases the termination of the spine shows a progressive decrease in the size of several vertebrae, reminding one of the pygostyle of a bird, or the os coccygis of tailless mammals. Sometimes several terminal vertebrae fuse. There are cases where the spine ends with a free fragment or with a partly luxated vertebra, or even with fragments reminding one of aberrant vertebrae originating from a more distal part of the tail.

In newborn tailless mice, according to our observations, lesions in the region of the sacrum are found frequently. In these cases blebs or haematomata are observed underneath the epidermis. These blebs represent cysts of spinal cord tissue. The spine itself terminates anterior to such a cyst and the last part of spinal cord which does not find any protecting vertebrae forms the cyst. In the adult, apparently, no trace of this lesion can be found externally. In a few extreme cases paralysis of the hind limbs has been observed in newborns, due probably to absence of nerves as a result of extreme shortening of the spinal cord. In dissection of one such case (29 line) no ventral roots were found in the last part of the spinal cord. This would account for the paralysis of the hind limbs. In two other cases (A and 29 line) two tailless mice were born without an anal opening. One of these newborns had also an abnormally small genital papilla.

DEVELOPMENT OF THE TAILLESS EMBRYO

For the study of the early stages of the embryogeny of the normal mouse up to the eighth day after fertilization three papers by SOBOTTA (1895, 1903, 1911) have been used. An excellent Normentafel was published recently by HENNEBERG (Keibel's Normentafel 1937) for the rat, in which development is extremely similar to that of the mouse.

The development of the tailless embryo proceeds normally up to the eleventh day after fertilization. The tail bud becomes distinct on the ninth day of development. It grows and lengthens and at about the end of the tenth day the tail of the normal mouse begins to grow very fast and length-

ens considerably (fig. 1a). The tail of the prospective tailless mouse also grows and lengthens rapidly but at the beginning of the eleventh day a sharp constriction sets in at the proximal end of the tail (fig. 1b). This constriction always occurs at the same level and demarcates the tail from the trunk. While on the tenth day of development the somite number in the tail of both the normal and the tailless mouse is the same (it averages 6) there is a marked difference on the eleventh day. At this time it averages twenty in the normal mouse and fifteen in the tailless mouse. These numbers indicate that the somites in both tails have increased in number, in the tailless mouse however to a lesser degree than in the normal mouse. On the twelfth day the somite number in the tail of the normal mouse averages twenty-seven, while that of the tailless mouse averages twenty-one. From the thirteenth day on, no somites are recognizable in the tail of the tailless mouse by gross examination.

From the eleventh day on, there is a marked difference in the appearance of the tail of the normal and that of the tailless mouse. The constriction at the proximal tail end becomes more and more apparent. While in the normal mouse the trunk and the tail continue in one unbroken line, there is a sharp dividing line between the trunk and the tail in the tailless mouse (fig. 2a and b).

The tail of the future tailless mouse reaches its maximal length on the twelfth day of development. From then on it decreases in length and thickness, and finally, on the fifteenth day, there is but a small filament left (fig. 3b). In most cases this filament persists during the lifetime of the mouse; in a few cases, however, it is resorbed, and the mouse is born with no trace of a tail whatsoever.

Irregularities of the tip of the developing tail are quite frequently observed in both the normal and the future tailless embryo. In two litters of normal mice which were used for controls—one dissected on the 13th day after fertilization, the other on the 14th day—embryos with forked tail tips were found. Forking of the tail tip was also observed frequently in tailless embryos (fig. 1b). It occurred in both normals and abnormals most frequently at the age of 13 or 14 days, at a time when the growth rate of the tail is very high.

The gross examination clearly demonstrates that the absence of the tail in the adult mouse is not due to a complete suppression of the anlage but that the tail of the future tailless mouse develops quite normally up to a certain point after which some process sets in which effects retrogression and resorption of the tail.

HISTOLOGICAL EXAMINATION OF THE TAILLESS EMBRYO

The histological examination of embryos makes it possible to diagnose

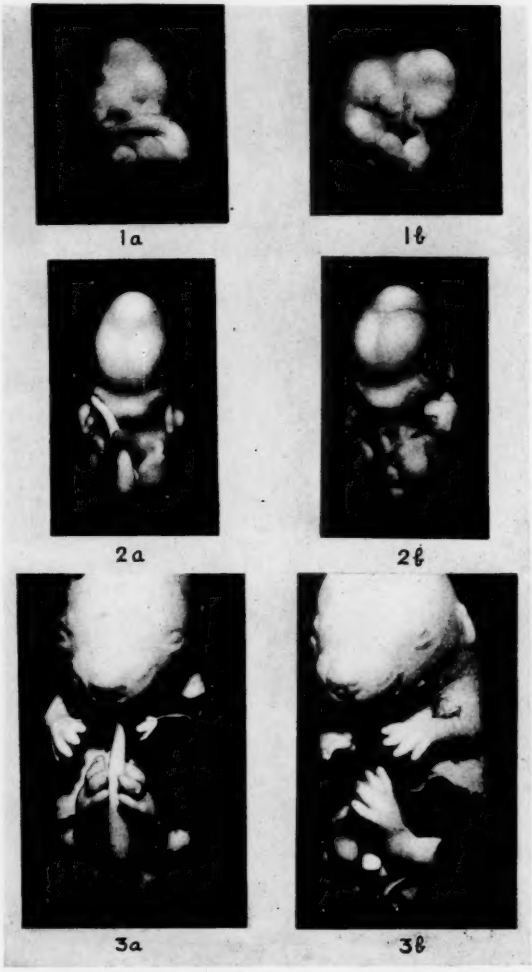
future taillessness one day before this diagnosis is possible on external criteria. So far no difference whatsoever in histological structure could be detected between the normal and the future tailless embryo before the tenth day of development. Further detailed histological and cytological studies might possibly reveal differences in certain organ systems before this time. The purpose of the following description is to demonstrate the histological differences that have been found between the normal and the tailless mouse from the tenth day of development on.

In a sagittal section of the normal embryo on the ninth day the following structures are to be found in the most posterior region of the trunk and in the tail bud. Dorsally the medullary tube reaches to the very tip of the tail bud. Ventral to it the notochord runs parallel to the medullary tube, but does not reach the very tail tip. Slightly anterior to the tip it opens up into the mesenchyme of the tail bud. Ventral to the notochord a sagittal section of the tail gut is found, the tail gut also running parallel to the medullary tube with the notochord thus taking the space between the medullary tube and the tail gut. The tail gut extends as far distally as the notochord and does not reach the tail tip. At this stage of development no difference could be found between normal and tailless embryos in any region. Among the litters examined a number came from tailless by tailless matings so that half of the offspring would be lethal (*TT* and *tt*) and the other half would be tailless. No difference could be detected between these tailless embryos and embryos from normal matings on the ninth day of development. In litters from matings of Brachy by heterozygous normal mice all embryos were alike up to the tenth day after fertilization.

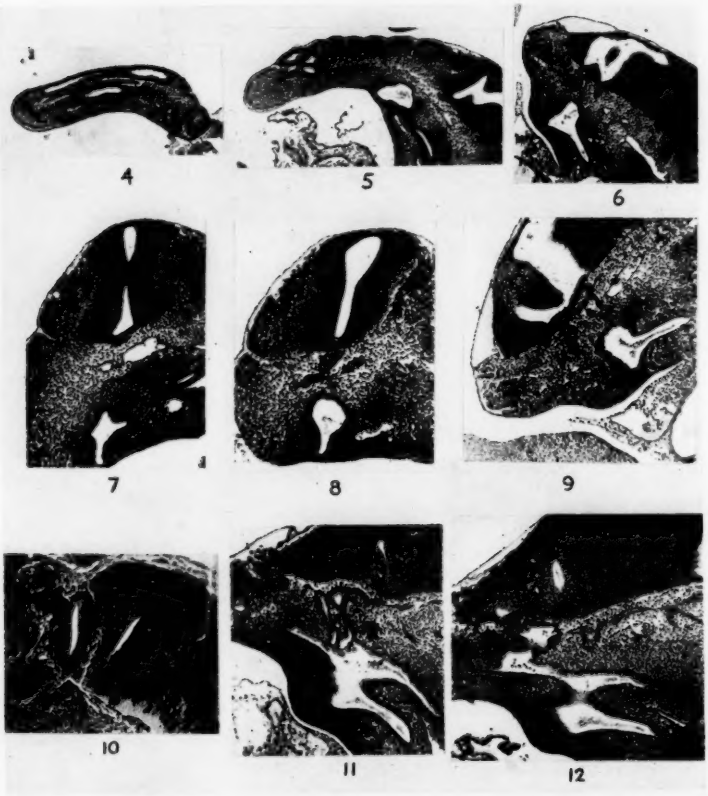
The first histological differences which can be detected between normal and tailless embryos are seen during the tenth day of development, that is, when the tail first begins to elongate. In the normal mouse a sagittal section of the tail contains the medullary tube reaching up to the very tail tip, the hind gut, and the notochord lying in the space between medullary tube and hind gut (fig. 4). In the tailless mouse no notochord is found in the tail. Sections of the neural tube are found in the tail; the neural tube sometimes is straight in the tail, sometimes it curves and bends. Sections of the hind gut in the tail show this structure to be normal at this stage, and regular somites are found in the tail of the mouse that is going to be tailless (fig. 5).

EXPLANATION OF FIGURES 1a–3b

1a) Normal embro. 2624, 3r. Age 11 days. Lateral view. 10×.
1b) Tailless embryo. Forked tail tip. Line 29. 2624, 4r. Litter mate of 1a. Lateral view. 10×.
2a) Normal embryo. 2502, 3l. Age 13 days. Ventral view. 10×.
2b) Tailless embryo. Line 29. 2502, 2l. Litter mate of 2a. Ventral view. 10×.
3a) Normal embryo. 2703, 2r. Age 15 days. Ventral view. 10×.
3b) Tailless embryo. Line 29. 2703, 1r. Litter mate of 3a. Ventral view. 10×.



FIGURES 1a-3b



FIGURES 4–12

There exists thus a striking difference between the tail bud and the elongated tail of the normal and of the mutant mouse. While in the normal mouse all structures present in the tail bud are drawn out into the elongated tail, the elongated tail of the future tailless mouse does not contain all of the structures present in the tail bud; it lacks the notochord. It seems that the notochord has reached its furthest posterior extension in the tail *bud* of the mutant embryo; it does not lengthen during further growth of the tail. The appearance of diverticula and ventral branches of the notochord in the posterior trunk region of the mutant (to be described below) seems to show that the notochord in its posterior part still possesses certain growth capacities although it does not grow into the elongated tail.

Apart from the tail, the most posterior part of the trunk also shows abnormalities. The structure most abnormal here is the notochord which in this region quite frequently sends off ventral branches. Figures 6 and 8 show such a ventral branch of notochord which cuts across the aorta and comes into contact with the epithelium of the cloaca. Furthermore, in the normal mouse embryo there is a wide space between the cloaca and the neural tube filled with mesenchyme (fig. 7). In the tailless mouse the space between cloaca and neural tube is very much smaller. The posterior trunk region seems to be compressed in a dorso-ventral direction (fig. 8).

Figures 11 and 12 show very abnormal conditions in the posterior trunk

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EXPLANATION OF FIGURES 4–12

4) Parasagittal section of tail of normal embryo. 3040, 3r. Age 10 days. Neural tube, notochord, hind gut.

5) Parasagittal section of tail of tailless embryo. 3040, 5l. Line 29. Age 10 days. Notochord entirely absent in tail. Neural tube, hind gut, somites. Last trace of notochord ventral to neural tube in posterior trunk region.

6) Oblique section of posterior trunk region of tailless embryo. 2624, 4r. Line 29. Age 11 days. Ventral branch of notochord in contact with epithelium of cloaca. Bleb between posterior end of neural tube and epidermis.

7) Oblique transverse section of posterior trunk region of normal embryo. 3040, 4l. Age 10 days. Notochord small rod ventral to neural tube.

8) Oblique transverse section of posterior trunk region of tailless embryo 3040, 11r. Age 10 days. Ventral branch of notochord below neural tube cuts across the aorta and comes into contact with epithelium of cloaca. Smaller space between neural tube and cloaca than in normal litter mate in figure 7.

9) Parasagittal section of posterior trunk region of tailless embryo 2624, 4r. Line 29. Age 11 days. (See figure 6.) Notochord running ventral to neural tube shows a number of branches and diverticula.

10) Parasagittal section of posterior end of neural tube of tailless embryo 2505, 11r. Line 29. Age 12 days. Neural tube bends laterally at its end. Diverticula of neural tube.

11) Parasagittal section of posterior trunk region of tailless embryo. 3233, 7l. Line A. Age 11 days. Fingerlike branches of hind gut that are in contact with the notochord which can be seen ventral to the neural tube.

12) Parsagittal section of posterior trunk region of tailless embryo 3233, 11r. Line A. Age 11 days. Notochord—ventral to neural tube—shows a number of ventral branches and is in contact with the highly malformed hind gut.

region of tailless embryos at the age of 11 days. Fingerlike branches of the hind gut establish a connection between hind gut and notochord while the notochord itself also sends off ventral branches.

In considering the abnormalities just mentioned, it should be remembered that during its development the notochord passes a stage at the age of 8 days when it is partly incorporated as chordal plate in the dorsal wall of the gut (HUBER 1918). This stage is followed by one in which the chordal plate again separates from the wall of the gut. The possibility exists that failure of the notochord to separate entirely from the gut might be responsible for the presence of the branches connecting notochord and hind gut, or notochord and cloaca.

On the eleventh and twelfth days of development the abnormalities in the tail and posterior trunk region of the tailless embryo become more and more marked. In sections of the constricted tail no notochord is found. For a short while, neural tube and gut, both not straight but curled and bent, are still to be found in the tail. Soon these structures disappear; they probably are resorbed. The last structures recognizable in the tail are the somites and blood vessels, and finally by the fourteenth day only mesenchyme and skin are left in the tail filament.

On the eleventh day the neural tube and the hind gut still extend further distally than does the notochord. The notochord in its posterior part sends off branches ventrally (fig. 9) which frequently come in contact with the cloaca. The last part of the notochord usually is not straight but bent ventrally. It may be forked at its end, and even the branches themselves sometimes are forked at the end. On the twelfth day the termination of the neural tube of the tailless embryo is found in the most distal part of the trunk. No filamentum terminale is present. The neural tube ends rather abruptly and often is not straight but curled. Frequently a bleb is found between the neural tube and the epidermis of the back (figs. 6 and 9). In the neighborhood of the termination of the neural tube small fragments of neural tube tissue are found in the surrounding mesenchyme. In embryo 2505, 1 r (fig. 10) a number of diverticula of the neural tube have formed at its posterior end.

The abnormalities in the posterior region of the trunk are very variable. The notochord is always abnormal, but it may have a variable number of branches which may or may not be in contact with the epithelium of the cloaca. Frequently connections between the notochord and the cloaca are established by fingerlike branches of the hind gut (figs. 11 and 12). The space between neural tube and cloaca, containing mesenchyme, may be more or less compressed. The termination of the neural tube in this region looks different in different animals. There may be some small fragments of neural tube tissue in the region of the end of the neural tube.

Sometimes a bleb forms dorsal to the neural tube and immediately beneath the skin. The variability in the tailless embryos corresponds to that in the tailless adults. There is no strict uniformity in the abnormalities of either.

DISCUSSION

The development of hereditary taillessness in two strains of mice has been described. Taillessness in these strains is due to the interaction of *T* (Brachyury gene) with either t^0 (A line) or t^1 (29 line). The development of the short-tailed mouse (Brachy heterozygote $T+$) has been described by CHESLEY (1935). There are a great many similarities in the development of the short-tailed and the tailless mouse. In both cases the tail is first formed normally and then later a constriction sets in, with a resulting resorption of that part of the tail and all the structures in it that lie distally to the point of constriction. In both the short-tailed and the tailless mouse the constriction appears on the eleventh day of development. The same kind of malformation of the notochord is observed in both of them, shortening of the notochord and the appearance of ventral branches in the posterior trunk region which come in contact with the gut epithelium. A difference between the heterozygous Brachy and the tailless mouse lies in the fact that in the short-tailed mouse the place varies at which the constriction sets in; the tail may become constricted anywhere between its proximal and its distal end. In the tailless mouse, however, the constriction always occurs exactly at the proximal end. Since in the adult tailless mouse the number of missing vertebrae in the sacro-caudal region varies, it follows that in the tailless embryo there must be a high degree of variability in the processes which take place in the reduction of the vertebral column. A special study of the development of the vertebral column in the tailless mouse has not yet been made. The high degree of variability in the posterior trunk region of the tailless embryo as far as the notochord is concerned has been mentioned above. However, the *place of tail constriction* is always the same in the tailless mouse.

This case of the tailless mouse reminds one also of certain wing mutants in Drosophila (GOLDSCHMIDT 1937). In both, development is normal up to a certain critical point. At this point in the tailless mouse, a resorption of the tail whose anlage had been perfectly normal, takes place. A degeneration of the tissue of the wing margin occurs in the mutant fly.

The present data do not give any answer to the question whether there is a time element involved in the different action of the Brachy heterozygote ($T+$) and the tailless heterozygote (Tt). One might expect at first to find that the process which produces complete taillessness sets in earlier than the process which produces a short tail. From external observations, however, it seems that the constrictions of the tail—that resulting

in taillessness as well as that leaving a short tail—set in at the same time, namely at eleven days. Since the short-tailed embryos (CHESLEY 1935) were timed within ±12 hours and the tailless embryos were timed within ±10 hours, it would be impossible to decide from the existing data whether a *small* time difference exists in the onset of the resorption process. Certainly no striking time difference was found. From histological observations also there is no evidence of a time difference in the onset of the two processes. CHESLEY found definite abnormalities of the notochord in heterozygous embryos at ten days, that is, before the appearance of a constriction in the tail. The same was found to be true in the tailless embryo at ten days, before any abnormalities could be detected grossly. From the data it seems as if the abnormalities in the notochord at ten days were greater in the future tailless embryo than they are in the short-tailed embryo. However no conclusive evidence of this exists, since from CHESLEY's data the exact extent of the notochordal abnormalities in the tenth day embryo is not clear.

The structures affected in the embryo in the tailless mutation are the neural tube, the notochord, the mesoderm in the tail and in the posterior trunk region, the cloaca (contact of its epithelium with the notochord). According to SOBOTTA the embryonic mesoderm in the mouse embryo arises from the wall of the primitive gut. The primitive gut of the mouse is a small blind canal about 30μ in length which runs obliquely from the cell mass of Hensen's node (at the anterior end of the primitive streak) toward the yolk sac cavity in an antero-ventral direction. It opens into the yolk sac cavity. The primitive gut in the mouse corresponds to the so-called neurenteric canal in other mammalian embryos (SOBOTTA 1911). Aside from the embryonic mesoderm, the wall of the primitive gut also gives rise to the notochord and to parts of the wall of the future gut. This seems to suggest that the malformations found in the tailless embryo might be traced back to some kind of malformation in the primitive gut region. Either some definite structure in the primitive gut has been affected by the mutation, or the primitive gut as a whole might have become affected at a certain time when it gave rise to the posterior part of the notochord, to the wall of the gut or the cloaca and to the mesoderm in the tail and posterior trunk region.

CHESLEY from his results concludes that "the abnormality of the notochord is one of the more fundamental of the disorders involved, and that the condition of the neural tube is either wholly or in part due to the abnormality of the notochord." CHESLEY further cites examples from the literature indicating the significance of the notochord for the development of the neural tube. Our data point in the same direction. The notochord

seems to be the structure first affected in the tail, malformations of the neural tube follow. While at the end of the tenth day of development the notochord of the mutant mouse differs from that of the normal by ending in the posterior trunk region and not extending into the tail, the neural tube of the mutant mouse is still normal at this time and extends into the elongated tail. Aside from this time difference in the onset of the developmental disturbances in the notochord and the neural tube, it ought to be noted that the elongated tail of the tailless mouse *never* contains any notochord, while it does contain neural tube up to the time when it becomes resorbed. Never were malformations of the neural tube found without simultaneous abnormalities in the notochord. In young stages, on the other hand, it is possible to find malformations in the notochord without associated abnormalities in the neural tube. Our data do not give *conclusive* evidence for conceiving the malformations of the neural tube as secondary to the disorders of the notochord, but they point in this direction.

If it were possible to establish definitely that abnormalities exist in the region of the primitive gut in early stages of future tailless mice, one would be justified in ascribing to these regions of the primitive gut the responsibility for the development of the malformed structures in the older embryos. At the same time one would be justified in drawing from these abnormal conditions conclusions on the causal relations of these same structures in normal development. Further work is being done in this direction.

Death of the homozygous lethal in the 29 line takes place either before or just at implantation. The homozygous lethals in the A line are found dead shortly after implantation, at the age of six days. The morphology and the development of these lethal types are being investigated.

SUMMARY

1) The development of tailless mice of two strains (line A and line 29) is described.

2) Embryos that are going to be tailless can be distinguished from their normal litter mates at the beginning of the 11th day after fertilization on gross examination, by a constriction that separates the tail from the trunk in the tailless embryos.

3) The tail which grows normally up to the 11th day retrogresses after the constriction has taken place and is then resorbed. By the 14th day only a small filament is left in place of the tail.

4) By histological examination it was found that the constricted tail contains somites, neural tube and hind gut, but no notochord on the 10th and 11th days after fertilization.

5) From the 12th day of development on, the tail filament contains no

neural tube and hind gut; soon the somites disappear also, and finally only mesenchyme is left in the filament.

6) The notochord is reduced in the posterior trunk region of the tailless embryos and sends off ventral branches. The neural tube is reduced also in the posterior trunk region and has diverticula. The space between neural tube and cloaca is smaller than in normals. No irregularities in the anterior body region were found in the tailless embryos.

7) From the present data it is not possible to conclude definitely that the notochord is the structure primarily affected in this mutation and the neural tube only secondarily. The results do, however, point in this direction.

8) The possibility that the abnormalities in the tailless mouse trace back to a malformation of the primitive gut region in an early stage of embryogeny is discussed.

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GENETIC AND ENDOCRINE STUDIES ON A TRANSPLANTABLE CARCINOMA OF THE OVARY

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Received July 2, 1938

INTRODUCTION

A CARCINOMA of the ovary has been maintained by successive transplantations in mice of the CBA strain for nearly three years. In the first study with this tumor, it was determined that only normal males of the CBA strain would show progressively growing tumors during a three-month period. It was also ascertained that the growing transplanted tumor secreted appreciable amounts of estrogen. The criteria of hormone secretion of the transplanted tumors were: (a) long continued estrous vaginal smears which, after removal of the tumors reverted to the diestrous type in castrate females; and (b) growth of the rudimentary mammary glands of males as the transplanted tumors grew. Insufficient hormone was secreted to affect the pelves (STRONG, GARDNER and HILL 1937).

The above conclusions reopened the problem of immunity to this transplanted neoplasm. The transplantation of neoplastic tissue (primarily based upon carcinoma of the mammary gland) demonstrated that the progressive growth of the tumor was determined by genetic factors inherited according to the accepted laws of mendelian heredity. No functional characteristic of the grafted tumors, however, has been demonstrated in these tumors growing in genetically controlled strains of mice. The presence of multiple factors underlying susceptibility and immunity to the previously grafted tumors has complicated the physiological factors that should have been evident. In the only case in which susceptibility and immunity to a grafted tumor was apparently controlled by a single mendelian factor, the especially selected strain of mice was lost before adequate physiological proof was obtained (STRONG 1926).

The present tumor, a carcinoma of the ovary, was a suitable tissue for our purpose. The sex difference encountered in the natural susceptibility or immunity to the tumor in inbred CBA mice suggested that a simple genetic mechanism was involved. Again, the hormone factors involved in sex physiology are so well known that ample opportunity of investigating

[1] This experiment has been made possible by grants from the International Cancer Research Foundation, the Anna Fuller Fund and the Fluid Research Funds of Yale University, School of Medicine.

this problem in relation to the grafted tumor should not be further delayed. The secretion of estrogen by the tumor during its growth as a transplant is a type of physiology which can be easily measured. Other hormone preparations known to influence (1) follicular development of the ovary (the tumor probably arose from granulosa cells) and (2) sex physiology, should therefore be investigated. Finally the action or interplay of genetic and hormonal agencies in building up the physiological behavior of the individual seems to be indicated.

RESULTS

The genetic factors involved in the successful growth of the grafted tumor were studied by (1) the inoculation into normal male and female mice of the CBA (STRONG 1936) strain, (2) males and females of the A strain (STRONG 1936a) and (3) the hybrids of the first two filial generations produced by crossing individuals of these two inbred strains.

Inoculation into normal CBA mice

One hundred and sixty-eight female and 153 male mice of this strain were inoculated with the tumor. All males grew the tissue progressively (100 percent susceptible). Only eight of the normal females grew the tissue,

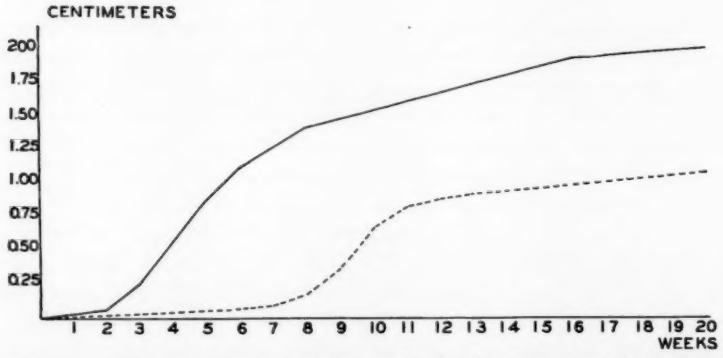

FIGURE 1.—The average growth rate of the transplant in (a) male mice (solid line) and (b) female mice (dash line) of the CBA strain. Time in weeks is plotted along the base line; size of tumor (greatest diameter in centimeters) is plotted along the vertical line.

and of these eight, seven occurred in the first two transfer generations. After a lapse of time (approximately a year) a new series of mice of the CBA strain were inoculated with the tumor and the females were kept for a considerable period after the males had all died with large tumors. Approximately eighteen percent of normal females of the new series eventually grew the tissue. In another experiment, a few female mice grew the

transplanted tissue to the size of a pea over a period of eighteen months. The sexual difference encountered was not an absolute one but rather one of degree. Whether more normal females of the CBA would later grow the tissue is of course not shown by the present data. The comparative analysis of susceptibility and resistance to the grafted tumor was significant only when the time relationships existing after the mice were actually inoculated were taken into consideration. The transplants grew much faster in males than in females (fig. 1). The tumors after a period of relatively rapid growth, were infiltrated with heavy calcified deposits, thus becoming rock-like, after which they did not increase much in size.

Results of inoculation into F_1 individuals

Since a sex difference was encountered in the inoculation of normal individuals of the CBA strain, the outcross to the A strain was made in both directions. Seventy-nine mice produced by crossing CBA females

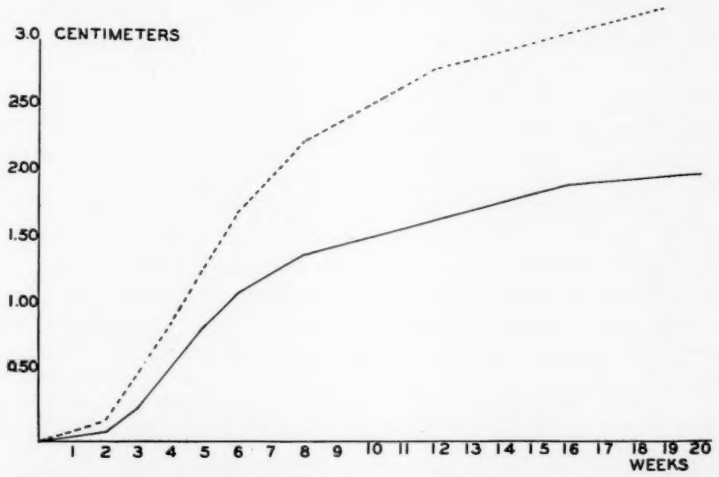

FIGURE 2.—The average growth rate of the tumor in (a) normal male mice of the CBA strain (solid line) and (b) F_1 male mice obtained by a cross between the CBA and A strains. Heterosis increases the growth rate of the grafted tumor.

with A males were inoculated with the tumor; of these, 26 were males and 53 females. All of the 26 males grew the tissue progressively; whereas only 10 of the 53 females grew the tumor. The percentage of female F_1 individuals growing the tissue (18.8%) was approximately the same as that obtained in purebred CBA females. In the other cross when A females were mated to CBA males, 91 mice were obtained. Of these, 44 were males and

47 females. All the males and 9 of the females grew the tumor. Of the F_1 females produced by this cross 21.3 percent thus grew the tumor or approximately the same proportion as that obtained in the first F_1 population and in the original CBA females. Apparently neither sex linkage nor sex limited inheritance was involved in susceptibility to this transplant. That both the males and females of the F_1 generation give the same percentage of susceptible individuals would indicate, however, that susceptibility to the transplanted tissue is intrinsically determined. The evidence indicates that a non-genic sex-limited influence is probably involved.

Male mice of the F_1 generation grew their tumors at a more rapid rate than male mice of the CBA strain, as indicated in figure 2. This finding has been encountered several times and indicates that heterosis is involved in the rate at which a transplanted tumor grows, as postulated by STRONG (1926a).

Results obtained in the F_2 generation

The data obtained by inoculating the tumor into F_2 individuals are presented in table 1.

TABLE 1

	CBA ♀ × A ♂			A ♀ × CBA ♂			AVERAGE PERCENT +
	+	−	PERCENT +	+	−	PERCENT +	
♀	8	148	5.1	16	196	7.5	6.5
♂	20	80	20.0	40	52	38.4	29.4

The data obtained in the male mice will be discussed first. It appears that multiple mendelian factors (probably four) are involved in the successful growth of this transplantable carcinoma of the ovary. The expectation for independent multiple factors in the F_2 generation is given in table 2.

TABLE 2

NO FACTORS	RATIO	PERCENT +
1	3:1	75.00
2	9:7	56.25
3	27:37	42.18
4	81:175	31.64
5	243:781	23.73

The percentage obtained (29.4) with this F_2 generation of male mice lies between that expected for four (31.64%+) or five (23.74%+) factors, nearer that expected for four factors.

The genetic analysis of susceptibility to the transplant for female mice of the same inbred strain was, however, not so clear. Definite ratios were

obtained both in pure strain individuals as well as in the outcross generations to pure strains thus indicating that intrinsic factors are involved in the process of transplantation. The same diminution of susceptibility is obtained in the F_2 individual females as was seen in a similar analysis for males in the same experiment. These comparative data are shown in table 3.

TABLE 3

	PERCENT +	
	♂	♀
CBA	100	18.2
F_1	100	20.1
F_2	29.4	6.5
$F_1 > F_2$	3.4	3.1

It is tentatively concluded, from the above analysis, that approximately four mendelian factors are involved in the successful transplantation of the carcinoma of the ovary in both males and females. The complete multiple-factor complex manifests itself in male mice only. Apparently some physiological mechanism within the female inhibits or prevents the full manifestation of its genetic constitution.

In order to test out this hypothetical mechanism that underlies susceptibility and resistance to the transplant an endocrinological investigation was undertaken.

STUDIES OF HORMONE TREATMENT ON TUMOR RESISTANCE

Effect of castration. Thirty-one male mice of the CBA strain, castrated at approximately six weeks of life, were inoculated with the tumor two weeks later. Of these, only fourteen grew the transplant. When the growth

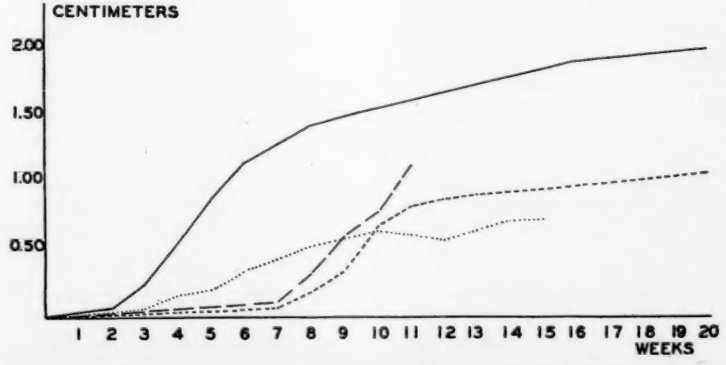

FIGURE 3.—The average growth rate of the transplant in (a) normal male mice (solid line), (b) normal female mice (long dash line), (c) castrate male mice (dotted line) and (d) male mice receiving estrogen (short dash line).

rate of the tumor is compared to that obtained in normal male and female mice of the same strain (fig. 3) it is seen that castration not only diminished incidence but also decreased the growth rate of the transplant similar to that obtained in normal female mice of the same strain.

Twelve female mice of the CBA strain were castrated at six weeks of age and inoculated with the transplant. Four of these grew the tissue progressively and slightly faster than did normal female mice of the same strain. Daily vaginal smears disclosed the presence of some estrogen but in insufficient amounts to keep the castrate female in continuous estrous.

Hormone injections. Since the incidence and growth rate of this transplanted ovarian carcinoma was related to the presence of the gonads, especially the testes, it might be affected by the sex hormones or gonadotropic hormones. This hypothesis was tested by injection of various hormones into 115 CBA mice in which the tumor had been transplanted under the skin in the region of the right axilla. Sixty females and 55 males were used, half of the females and 25 of the males were castrated. Five castrate and five non-castrate mice of each sex served as controls for the similar group which received daily injections respectively, of 25 i.u. of estrogen-2, (Folliculin Benzoate or Progynon B_2),[2] $\frac{1}{4}$ mg. Progestin (Prolutin and Progestin $\frac{1}{2}$ r.u. follicle stimulating hormone (Prephysin), 2.5 r.u. pregnant mare's serum (or Gonadin serum) and 0.05 cc. of egg albumen. The 0.05 cc. egg albumen was used as a control for the protein in the gonadotropic hormones. As Progestin had only a slight inhibitory effect on the growth rate of the tumor, it was used only in the normal male and female series, and 200 gamma (weekly) of male hormone (testosterone propionate-9) was used in the castrates.

RESULTS

The growth rate of the transplants in male mice receiving pregnant mare's serum was not affected (fig. 4). On the contrary, female mice receiving pregnant mare's serum grew their transplants at a more rapid rate than their controls (fig. 5). This enhanced growth rate may be due to (1) a "mutation" of the tumor similar to those encountered by STRONG

[2] 1. The Folliculin Benzoate was obtained from the British Drug House, London, England, and Progynon B was supplied through the courtesy of Dr. E. Schwenk of Scherring Corporation.

2. The Prolutein used was obtained through the courtesy of Dr. E. Schwenk of Scherring Corporation, and the Progestin through the courtesy of Dr. O. Kamm of the Parke-Davis Company.

3. The follicle stimulating hormone, Prephysin, was obtained from Chappel Bros., Inc., Rockford, Illinois.

4. The pregnant mare's serum was supplied by the Parke-Davis Company, through the courtesy of Dr. O. Kamm and the Gonadin was purchased from Cutter Laboratories, Berkeley, California.

5. Testosterone Propionate, "oreton," was supplied through the courtesy of Dr. E. Schwenk of the Scherring Corporation.

(1924, 1926b), BITTNER (1930, 1931) and CLOUDMAN (1932) with transplantable carcinomata of the mammary gland, or (2) the stimulation of the growth rate indirectly or directly without having undergone any cellular

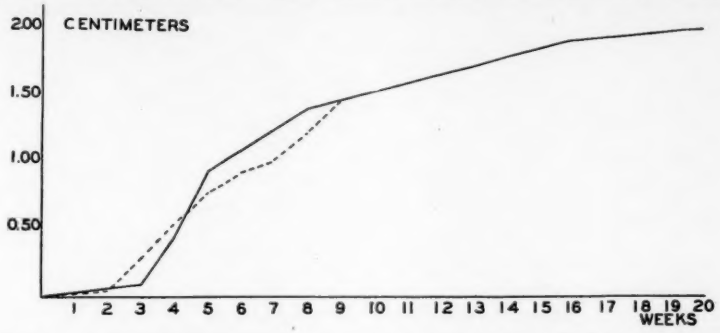

FIGURE 4.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving pregnant mare's serum (dash line). There is no difference between the two curves.

change. In order to test these two hypotheses, subsequent grafts were made from these enhanced growing tumors. The growth rate of these was the same as that obtained with the original tumor in male mice of the CBA strain. The second conclusion, therefore, seems to be the more logical one. In addition to a very rapidly growing tumor, one of the mice receiving pregnant mare serum also had several metastatic nodules in the lungs. This

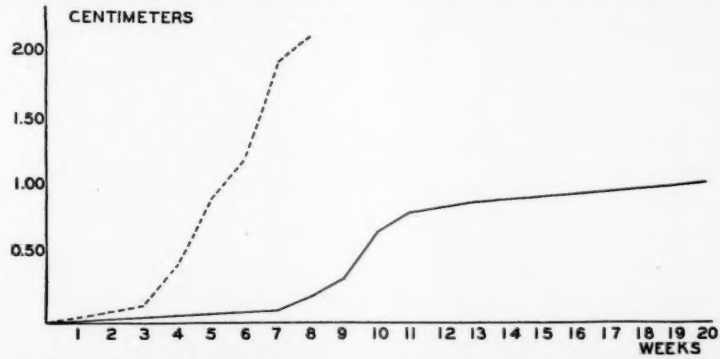

FIGURE 5.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving mare's serum (dash line). Female mice receiving pregnant mare's serum grow their tumors at an enhanced rate.

is the only mouse of several hundred examined that had a metastasis from this tumor. The tissue from the lung metastasis has been carried on by subsequent transplantations for four generations without any obvious

deviation from the growth rate of the original transplanted tumor, or evidence of further metastatic activity.

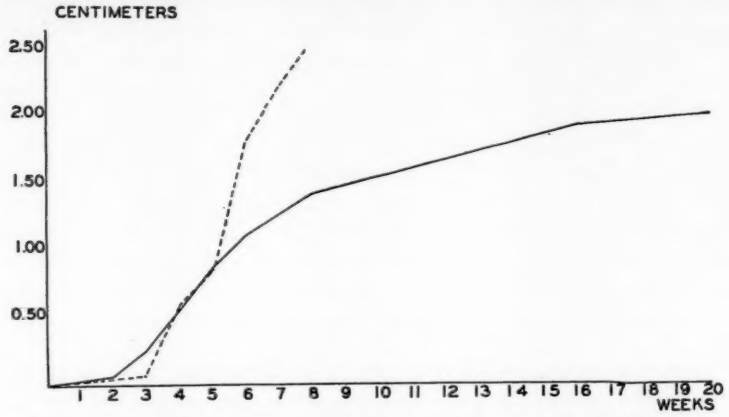

FIGURE 6.—The average growth rate of the transplant in (a) male mice (solid line) and (b) male mice receiving follicle-stimulating hormone (dash line).

The injection of the follicle-stimulating hormone had no detectible effect on the growth rate of the tumor or the survival time of normal female mice. On the other hand, it had a significant stimulating effect on the transplant growth in normal males (fig. 6).

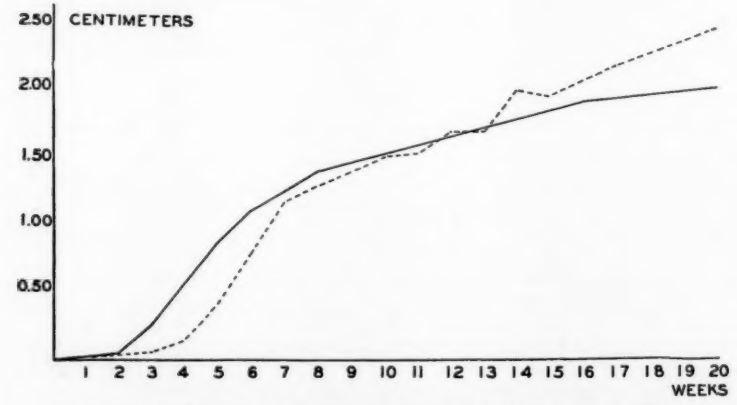

FIGURE 7.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving prolutin (dash line).

Prolutin may have had a slight inhibitory action on the growth of the carcinoma of the ovary, since the growth curve lagged behind that ob-

tained for the controls—and eventually approached the normal curve after the injections of prolutin were discontinued (fig. 7).

The injection of estrogen into both males and females inhibited the growth rate of the transplanted tumors (figs. 3 and 8).

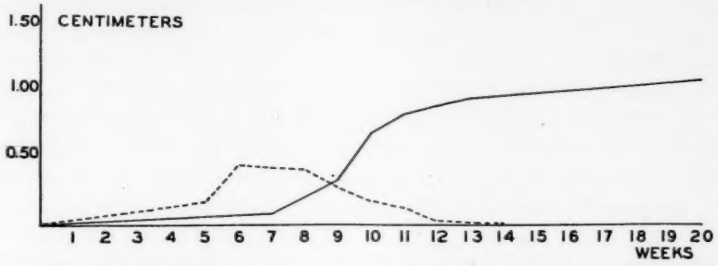

FIGURE 8.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving estrogen (dash line). Female mice receiving estrogen completely regressed their tumors.

The growth rate of the implanted tumor was not altered by the injection of any of the hormone preparations when the individual (male or female) had been previously castrated. The injection of egg albumen did not affect the growth rate of the tumor. The comparative effect obtained by the various injections is given in table 4.

TABLE 4

	FEMALE		MALE	
	NORMAL	CASTRATE	NORMAL	CASTRATE
Mare's Serum	+	o	o	o
F.S.H.	o	o	+	o
B.D.H.	−	o	−	o
Prolutin			−	
Egg Albumen	o	o	o	o
Male Hormone				o

GENERAL DISCUSSION

Though this tumor arose in the ovary a delayed or complete failure of its development following transplantation into intact female mice soon became apparent. Whether the tumor arose under altered hormonal conditions or underwent changes following transplantation afforded two possibilities for speculation.

Since the genetic study indicated a non-genic sex-limited regulation of transplantation and rate of growth the possibility of hormonal factors was investigated. The intact male afforded the optimum environment for the transplant; the intact female the least satisfactory host. Orchidectomy inhibited the growth of the tumor.

The increased rate of growth of the transplanted tumors in normal females receiving pregnant mare's serum might indicate that this effect was obtained by the action of the ovary. F.S.H. was, however, without effect. The failure of estrogen or progestin injections were not compatible with such interpretation. The lack of effect of pregnant mare's serum in intact males indicates an absence of any direct effect of this gonadotropic principle. The present experiments fail to explain definitely the role of endocrines indicated by the differences of growth of the transplant in the males and females. Some tumors particularly certain adenomas of genital tissues respond to their hormonal environment. The more malignant tumors have failed to respond to most hormonal deficiencies, presence or excesses, unless the general condition of the animal is greatly affected. It is particularly interesting that this comparatively malignant tumor has also retained to some extent the physiological activity of the tissue of origin.

The tendency of the tumor to calcify, particularly when growing slowly, in intact females also merits further investigation.

The genetic theory of transplantation of neoplastic tissue as postulated by STRONG formulated the conception that the fate of neoplastic tissue within the body was controlled by a reaction between the tumor and the host. The reactivity of the host is controlled by its intrinsic genetic constitution; that of the tumor is also determined by an internal genetic constitution. The data of STRONG, BITTNER and CLOUDMAN have clearly demonstrated that the genetic constitution of the tumor may change from time to time presumably by a process similar to somatic mutation.

The present data, on the other hand, demonstrate that the reactivity of the host toward a transplantable carcinoma of the ovary may be influenced by the injection of hormone solutions or by gonadectomy.

The genetic data demonstrate that susceptibility and resistance to the transplant are determined intrinsically by multiple mendelian factors. There are at least two hypotheses for the explanation of the relationship between genic and hormone action. The first one maintains that the early development of the individual is determined by genic action. This is eventually replaced by hormone action during the adult physiological life of the individual. The second hypothesis maintains that genic action and hormone action are both present simultaneously. With the accumulated data on the effect of genes throughout the entire life span of the individual and on the inheritance of disturbances of the endocrine system itself, it seems more likely that hormone action may very well be controlled by or be concomitant with genic action.

It is more than probable therefore, that in this investigation the influence of hormone preparations on susceptibility and resistance to a transplantable carcinoma of the ovary has been demonstrated by the possibility

that the defect or variation received by the individual through its germ plasm has been made good by hormone therapy.

SUMMARY

The growth of a transplanted carcinoma of the ovary depends on the simultaneous presence of multiple mendelian factors (probably four). The growth rate of the tumor can be influenced, however, by internal secretions of the host. However, the endocrine factors have not been clearly worked out because of (1) the extreme calcification undergone by the tumor and (2) the extremely slow growth of the transplant in intact females.

The problem of tumor susceptibility and resistance is discussed; the interest inheres primarily in the fact that the tumor has retained the physiological activity of the tissue from which it originated (that is, the ovary).

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SOME EFFECTS OF X-RADIATION ON THE NEUROBLAST CHROMOSOMES OF THE GRASSHOPPER, CHORTOPHAGA VIRIDIFASCIATA

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Received July 27, 1938

RECENT studies and interpretations of the genetic effects of X-ray induced translocations and inversions in plants and animals emphasize the need for additional information concerning the cytological aspects of such chromosome alterations. Realization of this has prompted a number of investigators (LEWITSKY and ARARATIAN 1931; MATHER and STONE 1933; STONE 1933; MATHER 1934, 1937; HUSKINS and HUNTER 1935; WHITE 1935, 1937; RILEY 1936; LEVAN 1936; GUSTAFSSON 1937; and NEBEL 1936, 1937) to undertake studies of the immediate effects of X-rays on chromosome morphology. Because the results of these studies, though agreeing in many essentials, nevertheless seem utterly opposed and contradictory in other respects, further work is needed, utilizing other and, as far as possible, more favorable material.

The neuroblasts of the embryo of the grasshopper, *Chortophaga viridifasciata*, were used in the present study. These cells are particularly suitable for observations of chromosome form and alteration (CARLSON 1937). They are present in relatively large numbers. They are readily identifiable because of their large size, which is sufficient to accommodate the metaphase and anaphase chromosomes with a minimum of crowding and overlapping; this is especially important in X-rayed material, in which one must be able to distinguish between translocation and juxtaposition. Also, like the better-known germ cell chromosomes of the grasshopper, these somatic chromosomes are large. In this species all are telomitic, and so have the form of simple rods. On the other hand, a disadvantage of this material, especially as compared with the microspores of certain plants, is the large number of chromosomes (23 and 24 in the male and female, respectively), which makes it impossible to identify and interpret changes in terms of individual chromosomes, after even a relatively slight X-ray treatment.

My thanks are due DR. A. F. BLAKESLEE for the opportunity of working at the Department of Genetics of the Carnegie Institution of Washington during the summers of 1937 and 1938. I am deeply indebted to DR. B. P. KAUFMANN and DR. M. DEMEREC for much valuable discussion as well as many helpful suggestions and criticisms relating to this study.

MATERIAL AND METHODS

Eggs containing embryos 3–5 weeks old were given X-ray dosages of 100, 125, 250, 500, 750, and 1000 r.[1] At the end of certain time intervals after irradiation embryos were removed from the eggs and made into permanent slides by BAUER'S (1936) modification of the aceto-carmine method. This consists of immersion for 30–40 minutes in aceto-carmine, spreading on an albuminized glass slip under a greased cover glass, removal of the cover glass in 95 percent alcohol, and mounting directly in euparal.

It is true that fixation and staining with aceto-carmine do not give the uniformly and consistently excellent chromosome preparations that many other technics do. The ease and speed that it affords, however, recommend its use where a large number of preparations must be made in a limited period of time, provided, of course, that the shortcomings of the method be kept in mind in interpreting results. The aceto-carmine method described above gives preparations that are adequate for the purposes of the present study.

EFFECTS ON MITOSIS

In the normally developing embryo at any given time different neuroblasts are in different stages of mitosis. Following irradiation there is a rapid disappearance of middle prophase through telophase stages. At the end of one and a half hours, after 500 r, only interphases and early prophases are left. This cessation of mitosis persists for a period of time varying with the X-ray dosage. Anaphases first begin to reappear after about 3, 7, 17, and 22 hours following dosages of 100, 250, 750, and 1000 r, respectively. Additional data on this effect will be included in a later paper.

The present study is based primarily on material fixed within a few hours of the end of the period of cessation, in order to obtain stages of the same mitotic cycle in which irradiation occurred. Since the mitotic cycle of the neuroblast requires at least two days, and probably often more, for its completion, alterations dealt with in the present paper are solely immediate effects.

CHROMOSOME ALTERATIONS

The findings of several investigators support the view that X-radiation may alter the chromosomes at any stage of the mitotic cycle. These changes are much more drastic, however, when the chromatin is diffuse, that is, in telophase through early prophase, than in stages in which it is

[1] In view of the recent conclusion of Gustafsson (1936, 1937) that in plant root tips the frequency of chromosome X-ray effects increases with the increase in water content of the tissues, it seems important to remark that all eggs used in the present study were kept saturated with water for at least several days previous to treatment.

concentrated in well-formed late prophase, metaphase, and anaphase chromosomes. MATHER and STONE (1933), WHITE (1935), RILEY (1936), and GUSTAFSSON (1936, 1937) found alterations in cells irradiated at interphase and early prophase stages. In addition RILEY reported breaks produced in late prophase and metaphase chromosomes. HUSKINS and HUNTER (1935) described chromosome and chromatid fragmentation and translocation in cells treated at telophase.

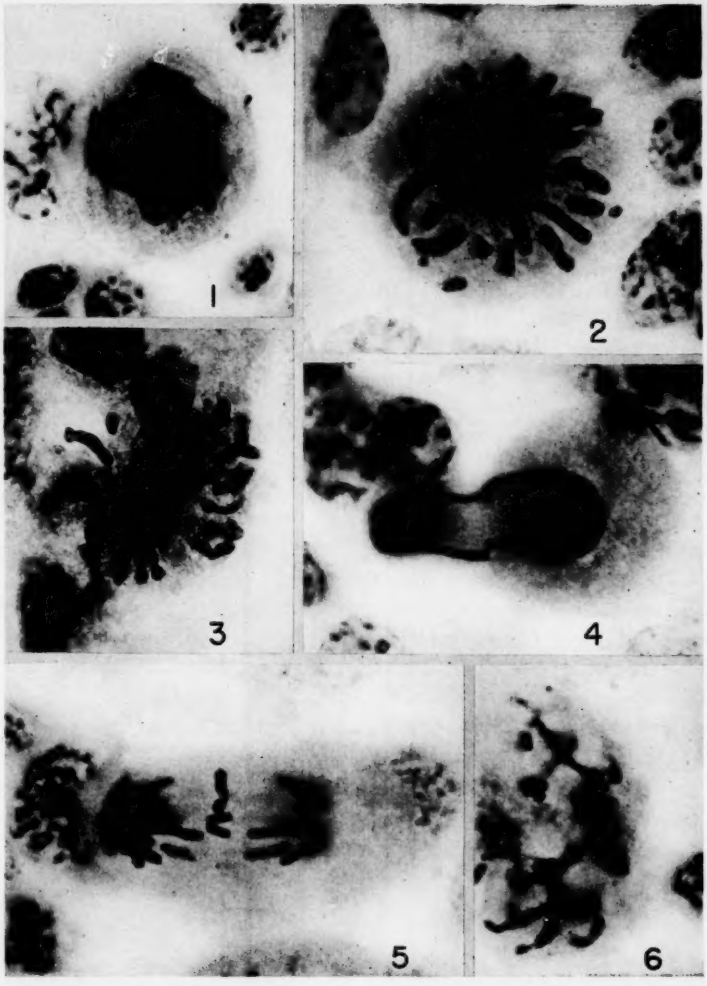
The chromosomes of mitotically active neuroblasts fixed within a few minutes after irradiation differ to some extent from normal, untreated ones. In late prophase sister chromatids appear to be very closely approximated at intervals along their length, these places having the appearance of constrictions. Metaphase chromosomes are longer than normal, exhibit severe constrictions, and show considerable twisting of sister chromatids about each other. Anaphase chromosomes appear joined together distally, while proximally they are elongated and show unusually broad constrictions (fig. 1).

X-ray effects that have occurred between telophase and mid-prophase, as observed in cells fixed in the subsequent metaphase and anaphase stages, show not only chromosome alterations but also chromatid and what may be half-chromatid inequalities. No evidence of either increase or decrease in the number of spindle fiber attachments was found in any of the cells in which accurate counts could be made.

In the following pages the term *fragment* is applied to a portion of a chromosome resulting from one or more breaks in an original chromosome and having no spindle fiber attachment. Either unaltered chromosomes or altered ones that have at least one spindle fiber attachment are referred to as *chromosomes*.

1. *Chromosome Fragmentation*

The fragments resulting from chromosome breakage are observable in the metaphase immediately following X-radiation. They are distinguishable from chromosomes with spindle fiber attachments, which are arranged with their kinetochores in an even circle and their distal ends extending outward, because they typically lie outside these chromosomes and have a less regular orientation (fig. 2). At early anaphase the "chromatids" of fragments, though lacking spindle attachments, separate at the same time as those of the chromosomes, which possess kinetochores. This separation may be complete or incomplete, but its occurrence proves conclusively that the initial anaphase separation of chromatids is entirely independent of the kinetochore (CARLSON 1938) and is probably autonomous. A more complete account of the behavior of fragments during mitosis is now in course of preparation.



FIGURES 1–6.—Photographs of irradiated neuroblast chromosomes. Length of time after irradiation: figure 1—about 3 min.; figure 5—12 hrs.; figures 3, 6—23 hrs.; figure 2—25 hrs.; figure 4—166 hrs. Dosage: figures 2, 4, 5—250 r; figure 1—500 r; figures 3, 6—750 r. ×1100.

FIGURE 1.—Anaphase. Pronounced constrictions present immediately after irradiation.

FIGURES 2, 3.—Metaphases. Fragments at periphery of cells. Note translocations in figure 3, one of which is shown in figure 16.

FIGURE 4.—Anaphase. Very long translocated chromosomes.

FIGURE 5.—Anaphase. Two V-shaped fragments in equatorial plane.

FIGURE 6.—Late prophase. Chromatid translocation. For details see figure 8.

The number of fragments per cell varies with the X-ray dosage. After 125 r there are rarely more than three or four fragments per cell, and frequently none at all. On the other hand, after 1000 r there may be more than thirty fragments per cell and no cells lacking them entirely. Because fusion of fragments *inter se* and with chromosomes obviously occurs with much frequency, counts of independent fragments give no accurate information of the actual amount of breakage originally effected by treatment, and so were not attempted.

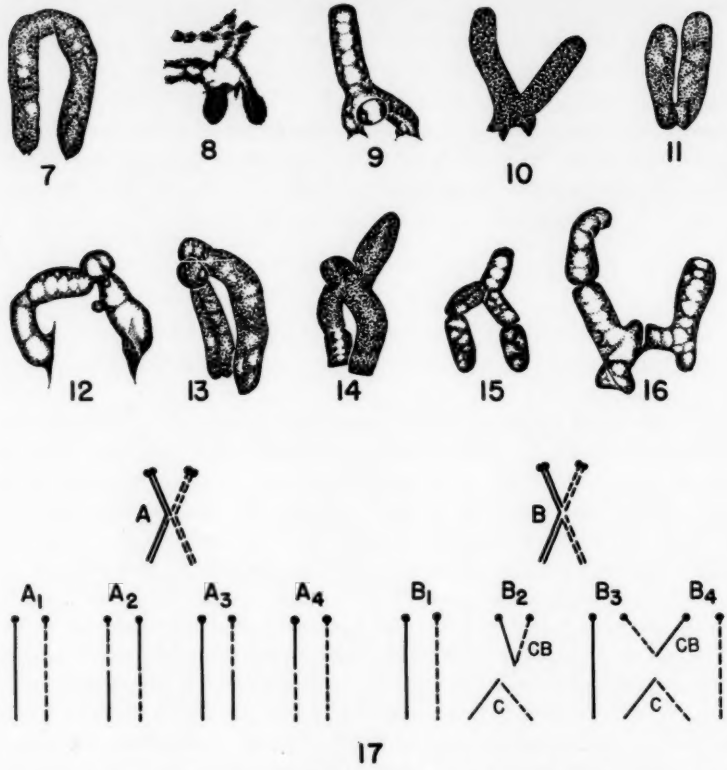
2. *Chromosome Translocation*

The large number of chromosomes per cell and lack of morphologically distinguishing characters make impossible any attempt to determine the number and extent of the chromosome translocations involved after irradiation.

Positive proof of translocation exists in the form of abnormally long chromosomes (fig. 4) and U-shaped chromosomes with two or more kinetochores (fig. 7). The anaphase behavior of the latter depends on the orientation of the kinetochores on the spindle (MATHER and STONE 1933; HUSTED 1936). If both spindle fiber loci of fused chromatids are directed toward the same pole, and the chromatids are not twisted about each other, a U-shaped daughter chromosome will move toward each pole (figs. 22, 23). If there is a half-turn in the chromosome, however, so that the spindle fiber loci of each fused chromatid point toward opposite poles, the anaphase configuration will have the form of two crossed chromatin bridges (figs. 24, 25). U-shaped chromosomes may persist as such, therefore, from one cell generation to the next, until they become oriented on the spindle with a half-turn from end to end. A delayed effect, resulting from the breakage of the crossed chromatin bridges and the subsequent formation of new attachments at the broken ends, might appear, as a consequence, a considerable number of cell generations after irradiation. The ultimate fate of chromatin bridges is considered in detail in a later section.

3. *Chromatid Inequalities*

Neuroblast chromosomes fixed and stained by most methods have, at metaphase and anaphase, a homogeneous appearance that affords no evidence of internal structure. In aceto-carmine smears, on the other hand, chromosomes in these stages usually exhibit a lightly staining "matrix" containing darker-staining regions comparable to the chromatids of the prophase (compare figs. 8 and 9). The metaphase chromosome and fragment each contains two such parallel regions (figs. 2, 3, 9, 12, 16, 26, 27). The anaphase chromosome and fragment each shows one—it may be double—lying in the middle of the chromosome and extending from end



FIGURES 7–17.—Irradiated chromosomes with more than one spindle attachment as a result of translocations. Spindle attachment ends, except in figure 17, are directed toward bottom of page. Figure 8 is in late prophase; others in metaphase. Length of time after irradiation: figures 13, 14—10 hrs.; figures 7, 15—12 hrs.; figures 11, 12—18 hrs.; figures 8, 9, 10, 16—23 hrs. Dosage: figures 1, 7, 8, 9—250 r; figure 5—500 r; figures 2, 3, 4, 6, 10—750 r. ×2475.

FIGURE 7.—Chromosome translocation.

FIGURES 8–16.—Chromatid translocations. Figures 8 and 16 appear as photographs in figures 6 and 3, respectively.

FIGURE 17.—Diagram of chromosomes with different types of chromatid translocations, showing the possible distribution of their chromatids at anaphase. A (diagram of figure 9) will give combinations A_1 and A_2 or A_3 and A_4 at anaphase. B (diagram of figures 6 and 8) will give combinations B_1 and B_2 or B_3 and B_4. CB, chromatin bridge. C, "chromatid" lacking spindle attachment. Knobs at ends of chromosomes and chromatids indicate positions of spindle attachments.

to end (figs. 5, 18, 28–31, 33–37). Although the appearance of these regions suggests that they are tightly coiled chromonemata, it seems best, until they have been examined after other treatment, to refer to them and the "matrix" around them non-committally as chromatids.

HUSKINS and HUNTER (1935) have demonstrated that some of the chromosomal constrictions so numerous in irradiated material are actually chromatid breaks. Certain X-ray induced constrictions present in my material appear to be the result of incomplete fusions of chromosomes, the ends of one chromatid of each chromosome having united and the other chromatids having remained unattached. A fragment of this type appears in figure 37. In the ensuing anaphase the distal portion of the interrupted chromatids will constitute a chromatid fragment (fig. 18 A). The non-corresponding constrictions exhibited by the daughter chromosome pairs shown in figures 18 A, 19, and 21 appear to be chromatid inequalities, but may possibly represent half-chromatid effects.

A frequently observed type of chromatid inequality in my material is what may be called a chromatid translocation (figs. 3, 8–17), namely, the "lateral translocation" of HUSKINS and HUNTER (1935), "reciprocal chromatid fusion" of WHITE (1935), or "pseudobivalent" of LEVAN (1937). The fusion may give either of two configurations. (1) If the proximal portion of one chromatid of each of the two chromosomes involved is joined to the distal portion of the other chromosome, each of the four chromatids will have a single kinetochore (figs. 9, 17 A), and the configuration will resemble the cross-shaped diakinetic tetrad. At anaphase two chromatids will pass to each pole, the orientation of the kinetochores on the spindle determining whether both (fig. 17, A_1 and A_2) or neither (A_3 and A_4) of the daughter cells will get a complete complement of chromosomes. (2) If the two proximal and the two distal portions are joined *inter se* at the point of breakage, the chromosome will consist of two unaltered chromatids, one chromatid with two kinetochores, and one with none (figs. 6, 8, 17 B). If the unaltered chromatids pass to the same pole (fig. 17 B_1), the one with the two kinetochores will pass to the other pole (B_2); but if the former pass to opposite poles (B_3 and B_4), the kinetochores of the latter will also move toward opposite poles, thus forming a chromatin bridge (CB). In each case the "chromatid" without a kinetochore (C) may pass into either of the daughter cells. Only one of these four possible daughter cells will possess a complete set of chromosomes, namely, the one into which the two unaltered chromatids pass.

4. Half-chromatid Inequalities

NEBEL and RUTTLE have concluded from observations of the somatic chromosomes of Tradescantia (1935, 1936) and Hordeum, Secale, and Crocus (1937) that doubling of the chromonemata occurs two generations in advance of their anaphase separation, so that from one anaphase through the next prophase each chromatid is composed of two chromonemata or half-chromatids. I have found a few figures in my prepara-

tions that seem very suggestive of half-chromatid effects, though none furnishes what I consider to be entirely convincing proof of the occurrence of such inequalities. This is due in part to the nature of this problem, itself, and in part to the fact that I have looked for these only in stages of the division immediately following irradiation, while the presence or absence of half-chromatid effects should be more readily detectable in the second division following irradiation.

In figures 18 A, 19, and 21 are shown anaphase pairs of daughter chromosomes that were irradiated in the interphase or early prophase condition. They exhibit constrictions at non-corresponding places. That such constrictions are not comparable to the secondary constrictions normally present in untreated material of many different organisms is evident from the fact that, in the latter, sister chromatids are constricted at corresponding points. They may, however, be comparable to the constrictions in metaphase chromosomes resulting from a break in one of the two chromatids (HUSKINS and HUNTER 1935), but, because they occur in anaphase instead of metaphase chromosomes, they would represent, by analogy, half-chromatid breaks instead of chromatid breaks.

One member of each of pairs A and B (fig. 18) displays a short region of abnormally small diameter. Comparison of the lengths of each of these with its sister chromosome suggests that the segment of small diameter has been inserted, its small size being due, perhaps, to the possibility that it is an inserted half-chromatid.

The lower chromosome of pair A (fig. 18) and one "chromatid" of the fragment shown in figure 34 have small blobs of chromatin protruding from the side. Underneath each and corresponding to it closely in size is a lightly staining area. It seems not unlikely that each little blob may be a piece of a half-chromatid that has been "knocked out" of the central part of the chromatid, but has remained connected with it peripherally.

CHROMATIN BRIDGES AND FRAGMENTS

The fact that X-ray studies of Drosophila salivary gland chromosomes have as yet failed to disclose a single positive case of a terminal inversion has led many to hold that a broken end of a chromosome cannot exist as such, at least over the course of many cell generations, and that, therefore, if a single break occurs, it is somehow eliminated. It is generally assumed that some kind of unsatisfied attraction causes broken ends of chromosomes to unite with one another. In the cell shown in figure 2 an original set of 23 chromosomes with 46 ends has been altered by irradiation to give a total of 26 chromosomes and fragments possessing together 52 ends. This and many other similar examples leave no doubt that new ends, whether of chromosomes or fragments or both, may be formed as a result of X-radiation.

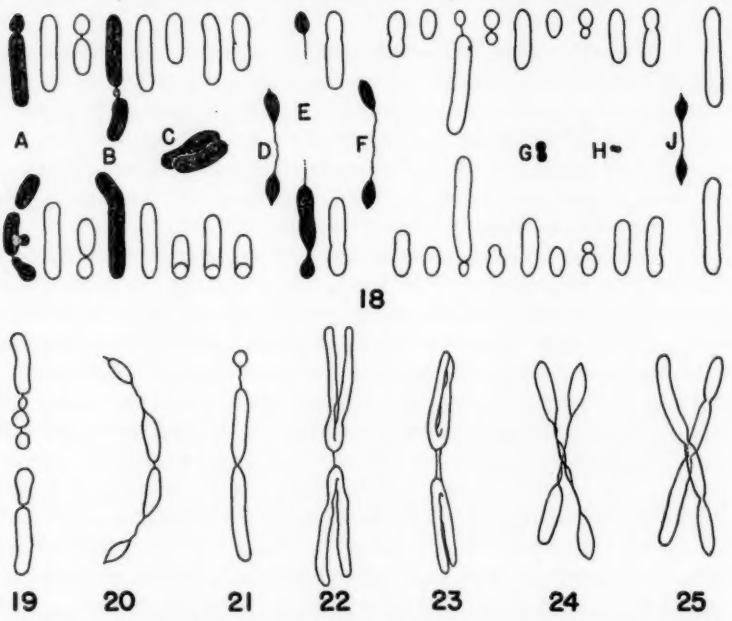
My material suggests, however, that the presence of such new ends does not actually demonstrate the existence of new, unsaturated ends of gene strings. It appears, instead, that in these instances the broken ends of sister chromatids of chromosomes and fragments have fused *inter se*. This assumption makes understandable two classes of chromosomal forms observed at anaphase, namely, chromatin bridges and fragments of three kinds.

A lagging at anaphase of certain daughter chromosomes and great attenuation of their distal ends to form chromatin bridges (fig. 18, D, F, J) result from failure of these ends to separate normally. It seems probable that the distal ends of the chromatids of such a chromosome are newly formed ends, which, unable to satisfy their attraction for broken ends of the chromatids of another chromosome, have fused with one another and tend to retain this union even at anaphase (fig. 38 CB).

The chromatin bridges shown in figure 18 (D, F, J) and in figure 20 may be expected to part at the site of the former break, so that equal daughter chromosomes with the same broken ends will pass into different daughter cells. If in the next cell generation the broken ends of the sister chromatids are again fused, chromatin bridges will be formed anew. Such broken ends can certainly, therefore, be transmitted from one cell generation to the next and may be supposed to persist in this manner from cell generation to cell generation until one of the following events occurs. (1) The broken end may unite with the broken end of a fragment at some subsequent cell generation to produce a normally behaving translocated chromosome (the "delayed attachment" of STADLER 1932). This presupposes that fragments may pass from parent to daughter cells, and this frequently occurs in these cells (CARLSON 1938). (2) The broken end may unite with the broken end of another chromosome to form a U-shaped chromosome with two spindle attachments. The mitotic behavior and probable ultimate fate of these has been described above. (3) The broken end may eventually acquire the properties of a true end, and so the chromatids cease to fuse at their broken ends to form chromatin bridges. Such chromosomes will then become normally behaving chromosomes with terminal deficiencies. This possibility is suggested by the X-chromosome deficiencies described by DEMEREC and HOOVER (1936) in Drosophila. (4) Sufficient genic material may be lost in the remnants of the chromatin bridge left outside the nucleus at the telophase of several succeeding divisions to produce a deficiency that is lethal to the cells. (5) The point of breakage of the chromatin bridge does not always occur at the same place, that is, at the point of chromatid fusion (fig. 18 E, 21), so that a deficiency is produced in one cell and a duplication in the other. Either or both may be cell lethal. The importance of any of these changes from the

genetic aspect will, of course, depend on whether or not the cell survives them; for, if it does not, the altered chromosomes will be lost with the cell.

Chromatin bridges have been observed in a number of different organisms in the first meiotic anaphase. They have been interpreted as chromatids with two spindle fiber loci resulting from crossing over in the inverted



FIGURES 18–25.—Anaphase separation of daughter chromosomes following irradiation. Length of time after irradiation: figures 19, 21—12 hrs.; figure 20—22 hrs.; figure 18—23 hrs.; figure 24—47 hrs.; figure 22—75 hrs.; figure 25—119 hrs.; figure 23—143 hrs. Dosage: figures 18, 19, 21–25—250 r; figure 20—500 r. ×1585.

FIGURE 18.—Complete set of anaphase chromosomes and fragments. A and B, chromatid or half-chromatid (?) inequalities. C, G, H, fragments. D, E, F, J, chromatin bridges.

FIGURE 19.—Chromatid or half-chromatid (?) inequality.

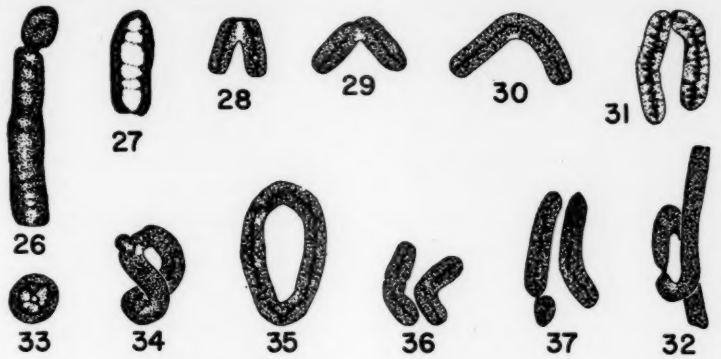
FIGURE 20.—Chromatin bridge.

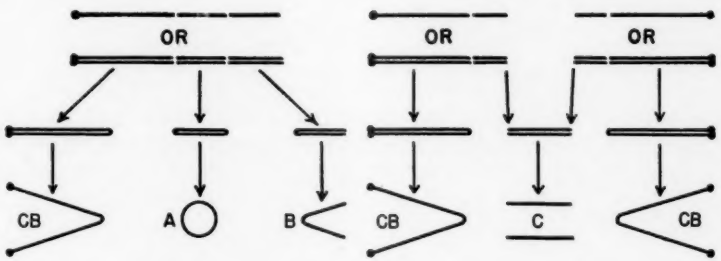
FIGURE 21.—Chromatid or half-chromatid (?) inequality and chromatin bridge.

FIGURES 22–25.—Types of separation of chromosomes with two spindle attachments.

portion of chromosomes in inversion heterozygotes. In the microspores studied by HUSTED (1936) and in my material, however, another explanation has been necessary to account for these chromatin bridges, since synapsis and crossing over cannot have been involved. To sum up, the conclusion seems justifiable that the chromatin bridges in my material have arisen in three different ways: (1) from chromsome translocation,

(2) from chromatid translocation, and (3) from fusion of sister chromatids at their broken ends.





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FIGURES 26–38.—Autonomous separation of "chromatids" of X-ray produced fragments, which lack spindle attachments. Length of time after irradiation: figures 27–32, 35, 36—12 hrs.; figure 26—13 hrs.; figures 33, 37—21 hrs.; figure 34—23 hrs. Dosage: figures 26–32, 35, 36—250 r; figures 33, 34, 37—750 r. ×2475.

FIGURE 26.—Metaphase fragment.

FIGURE 27.—Early anaphase. Beginning of separation of "chromatids" except at one end.

FIGURES 28–32.—Middle anaphase. V-shaped fragments resulting from "chromatid" separation except at one end. See figure 38 B.

FIGURES 33–35.—Anaphase. Ring-shaped fragments resulting from separation of "chromatids" except at both ends. See figure 38 A.

FIGURES 36, 37.—Anaphase. Complete separation of "chromatids" to give two rod-shaped fragments. See figure 38 C.

FIGURE 38.—Diagram showing types of breakage and fusion that lead to formation at anaphase of chromatin bridges (CB) and fragments having the form of V's (B), rings (A), and double rods (C). Knobs at ends of chromosomes and chromatids indicate location of spindle attachments.

During early anaphase the "chromatids" of fragments may behave in any of three different ways[2] (CARLSON 1938). First, they may separate

[2] This classification does not include the small spherical fragments that are present in most cells after treatment.

except at one end to form a V (figs. 5, 27–32). Second, they may remain connected at both ends and separate centrally to form a ring (figs. 33–35). Third, they may separate completely to form two rods lying side by side (figs. 36, 37). The fact that the "chromatids" of fragments, which lack kinetochores, begin to separate with the advent of anaphase, at the same time as the chromosomes with kinetochores, indicates that separation is an intrinsic character of the chromosome. Their tendency to remain attached frequently at either one or both ends, however, points not only to some fundamental difference in the ends as compared with the central parts of the fragments, but also to differences in the ends of the fragments. A given fragment end may be either the original end of the chromosome of which it was once a part, or a new one created in the breakage of the original chromosome at that point. The "chromatids" of a terminal fragment would be expected to fuse with one another at their broken ends. At anaphase these ends would remain fused, as the opposite true ends separated, to give a V-shaped fragment (fig. 38 B). Fusion would be expected to occur at both ends of an intercalary fragment, and the retention of this union after the central portion of the "chromatids" had separated would give the ring-shaped anaphase fragment (fig. 38 A). Finally, if a given fragment were the result of fusion at the broken ends of the terminal fragments of two original chromosomes, the remaining ends would be true ends, and so the "chromatids" would be expected to separate completely at anaphase to form two distinct rods (fig. 38 C). V's are the most abundant type. Next in frequency are the two rods. Rings occur only rarely at dosages below 500 r, but frequently at 750 and 1000 r.

In figure 18 three fragments (C, G, H) and four chromatin bridges (D, E, F, J) appear. According to the above hypothesis they would be explained thus. Fragments G and H are assumed to represent the true ends of two of the chromosomes connected by bridges. Fragment C is separating as two rod-shaped chromatid fragments. Both ends are, therefore, true ends, having resulted from the fusion, at their broken ends, of two distal fragments from the other two chromosomes with bridges.

UPCOTT (1937) and BARBER (1938) have reported chromatin bridges in microspores lacking fragments. The latter has shown, however, that aging of the pollen somehow causes a fusion of true ends of sister chromatids.

DISCUSSION

Time of chromosome doubling. The conclusions of different cytologists regarding the time of doubling, or splitting, of the chromosomes have recently been summarized by GUSTAFSSON (1936) and KAUFMANN (1936). Views range all the way from doubling in the late interphase or early pro-

phase immediately preceding anaphase to doubling in the prophase one mitotic cycle in advance of anaphase separation. Most of the chromosomes on which my results are based were probably in late interphase or early prophase at the time of X-radiation, and both chromosome and chromatid effects were produced. If the half-chromatid effects suggested by my material are valid, doubling has occurred in the late interphase or early prophase one mitotic cycle in advance of anaphase separation of the units thus formed. If one disregards these, however, there remains no important evidence bearing on this problem.

Mechanism of chromosomal change. According to the hypothesis proposed by SEREBROVSKY (1929) X-rays effect structural changes in the chromosomes by causing fusions of different chromosomes or different parts of a single chromosome, which is followed by breakage in a different plane. The proposal of STADLER (1932) is just the reverse of this; fusion is supposed to follow breakage rather than precede it. If it may be assumed that in either hypothesis the first occurrence conditions the second, an increase in the total number of chromosomal elements, namely, chromosomes and fragments, which is invariably the situation in cells showing any effects at all, seems to support the concept of breakage preceding fusion. In none of the affected cells in my material is there any decrease in the number of chromosomal elements, which would result if there were a predominance of fusion over breakage. Another difficulty of the SEREBROVSKY hypothesis is its dependence on close proximity or contacts between chromosomes at the time of effective irradiation, that is, between late telophase and early prophase. This requirement may be realized in many types of cells. The grasshopper neuroblast, however, is an unusually large cell, containing a large lobed nucleus with a central cytoplasmic core. The ends of three or four of the longer chromosomes extend distally into each of five or six lobes. Contacts between different chromosomes, therefore, are considerably limited. It seems improbable that a sufficient number of contacts could exist between these chromosomes at the time of irradiation to account for the complex fusion configurations present in many of these cells after treatment with 1000 r, unless it is assumed that treatment, itself, causes extreme movement of the chromosomes with the establishment of new contacts as treatment progresses. Of particular interest in this connection is a questionable translocation involving the X-chromosome and an autosome in figure 15 of WHITE'S 1935 paper on Locusta. The X chromosomes of many Acrididae, and, therefore, probably of Locusta, lie in vesicles apart from the other chromosomes from telophase through late prophase. The irradiation bringing about this translocation doubtless occurred during this period (this cell was fixed 17 hours after treatment),

and breakage could have occurred at that time. Fusion with the autosomal piece must have occurred subsequently, however, after the disappearance of the membrane of the vesicle at late prophase.

SUMMARY

The neuroblasts of grasshopper embryos treated with 100, 125, 250, 500, 750, and 1000 r suffer a cessation of mitosis for a period of time proportional to the dosage.

Cells irradiated between telophase and early prophase show, in succeeding stages of the same mitotic cycle, chromosome fragmentation and translocation, chromatid breakage and translocation, and what may be half-chromatid effects.

Chromatin bridges at anaphase appear to result from any of three different alterations: (1) chromosome translocation, (2) chromatid translocation, (3) fusion of sister chromatids of the proximal portions of fragmented chromosomes at their broken ends.

Chromatin bridge formation and the persistence of broken ends of chromosomes from one cell generation to another makes possible delayed reattachments following irradiation.

The early anaphase separation of chromatids, judged on the basis of the behavior of fragments lacking spindle attachments, is entirely independent of the kinetochore, and so is probably an autonomous function of the chromosome.

An hypothesis is suggested to account for three different forms, namely, V's, rings, and rods, which "chromatids" of these fragments assume as they begin to separate at early anaphase.

Certain evidence suggests that chromosomal change is effected by fusion following, rather than preceding, breakage.

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X-RAY INDUCED CHROMOSOMAL ALTERATIONS IN DROSOPHILA MELANOGASTER

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Received July 28, 1938

IN THE fall of 1936 the first two authors began a series of experiments to determine the frequency of minute inversions in material treated by X-rays. Before long it was realized that the frequency of such inversions is very low and that a reliable analysis would involve such technical difficulties that it would require a great deal more time than was available to the authors. Therefore, the original plan was revised and a new experiment started to determine the relation between X-ray dosage and the frequency of chromosomal breaks, the distribution of breaks along the chromosomes, and related problems. Subsequently when the experimental part of the problem was well under way the third author joined in the work.

The first author is responsible for the cytological analysis of the major portion of the female material, the second author was in charge of breeding and X-raying work and helped with the cytological analysis, and the third author is responsible for the cytological analysis of all male material and of a portion of the female data where Swedish-b females were mated with treated Oregon-R males. Permanent preparations of 1765 pairs of salivary glands were used for this study. Slides were prepared by Mr. HERSCHEL ROMAN, Miss RUTH BATE, and Miss EUNICE WHITE, to whom the authors wish to express their appreciation.

When this manuscript was ready for press a paper by CATCHESIDE (1938) appeared describing some of his results obtained in experiments identical with ours. Many conclusions reached by CATCHESIDE are identical with our conclusions.

MATERIAL AND METHODS

The bulk of the data reported here was obtained from experiments in which inbred Oregon-R wild type stock was used. However, before this experiment was completed a female sterility factor appeared in the stock and to avoid difficulties connected with sterility, females of an inbred Swedish-b wild type line were used in later experiments. In the chart (fig. 1) showing the relationship between X-ray dosage and effects, the data from Oregon-R females are presented separately because differences may exist in the genetic constitution of the females used. In analyses of other problems such differences would probably be unimportant and,

therefore, data from the two sets of experiments were considered together. The X-ray treatment was given to males and they were of the Oregon-R line in all experiments.

The X-ray radiation was applied by an Universal Type Coolidge tube, with a tungsten target, at 85 kilovolts and 7 milliamperes. The X-ray dosage was measured by a Fricke-Glasser dosimeter, manufactured by the Victoreen Instrument Company. In this work a 1/20 cubic centimeter graphite chamber was used by which the total dose applied was measured.

The study was made on salivary gland chromosomes of F_1 offspring from matings between untreated females and treated males. In all cases permanent preparations were made following in general the alcohol-euparal method outlined by BAUER (1936). One individual was available in each instance and in a great majority of the cases that material was sufficient to complete the analysis.

All chromosomal rearrangements were analyzed and breakage points determined within the limits of at least one division of BRIDGES' (1935) map. Small deficiencies involving one division or less were disregarded since they are easily missed in the material available in these studies. There are, however, two types of rearrangements not readily detectable by the method used, namely inversions and reciprocal translocations with both breaks within heterochromatic regions. Frequently unpaired basal sections may be found in non-corresponding chromosome limbs (for example, 2L and 3L) and this may be taken as evidence of such reciprocal translocation. As, however, the closeness of pairing may differ in different individuals, the possibility of detecting such cases is not always granted and their partial inclusion may lead to errors. As there is no reason to suppose that rearrangements of this type behave differently than other rearrangements, they have been omitted completely.

It has not been possible to determine the exact break number in all cases. In one type of altered sperm the aberration consists in the transposition of the nucleolus to a new place somewhere on the chromosome limbs. This probably represents an insertion of the nucleolus-bearing heterochromatic segment of the X or Y chromosome in another position, demanding three breaks (KAUFMANN 1938). Another type of configuration shows a terminal segment of one of the chromosomes attached to the chromocenter. In sperms having the Y chromosome such configurations have usually been analyzed as translocations involving that chromosome. In sperms having the X chromosome the rearrangement possibly represents a translocation involving those proximal ends of the X or fourth chromosomes which correspond to a second "limb" separated from the main body by the spindle fiber attachment as described by KAUFMANN (1934). On the other hand, it cannot always be excluded with certainty

that such cases represent translocations to chromocentral regions of the larger autosomes. These types of changed sperms have been counted as 2-break cases. In other larvae the complete determination of all breaks has not been possible, due to too few clear nuclei, and partly to the combination of changes of the unanalyzable type with other rearrangements. These have been classified for the lowest certain break number. In table 1 they have been registered in brackets behind the total numbers of cases in the respective classes.

ANALYSIS OF RESULTS

Number of breaks.—The number of breaks per changed sperm varies from 2 to 13. No case has been found with one break only which would

TABLE 1

Number of sperms with chromosomal aberrations.

NUMBER OF BREAKS	1000 r	2000 r	3000 r	4000 r	5000 r
2	8	16 (1)	128	38	47 (3)
3	3	4	22 (1)	8 (1)	9
4	—	3	25 (2)	12	14 (1)
5	—	1	8 (1)	1	9 (1)
6	—	—	5	2 (1)	2
7	—	—	1	—	4 (1)
8	—	—	—	1 (1)	1
9	—	—	1 (1)	1 (1)	—
10	—	—	1	—	—
Undeterminable	1	—	2	1	1
Total	12	24	193	64	87
Normal sperms	319	253	532	151	130
Grand total	331	277	725	215	217
% sperms with chromosomal aberrations	3.63	8.66	26.62	29.8	40.09
S. E.	±1.03	±1.69	±1.64	±3.12	±3.33
Breaks per changed sperm	2.250	2.542	2.704	2.875	3.126
S. E.	±0.125	±0.177	±0.092	±0.180	±0.164
% breaks per total of sperms	8.157	22.022	72.000	85.581	125.346
S. E.	±0.024	±0.045	±0.051	±0.105	±0.123

produce a terminal inversion or a terminal translocation to an unbroken end. Short terminal deficiencies, which might have been viable, have not been observed either. A special experiment was performed crossing Oregon-R males treated with 3000 r-units to attached-X females and studying triple-X female larvae. In addition to the treated X chromosome

such larvae had two untreated ones so that any terminal deficiency induced by treatment would be able to survive. Among 124 larvae examined, none carried a terminal deficiency.

Relation between dosage and frequency of breaks.—The relation between the dosage and the percentage of sperm with chromosomal aberrations was determined for 1000 to 5000 r-units at 1000 intervals. A summary of

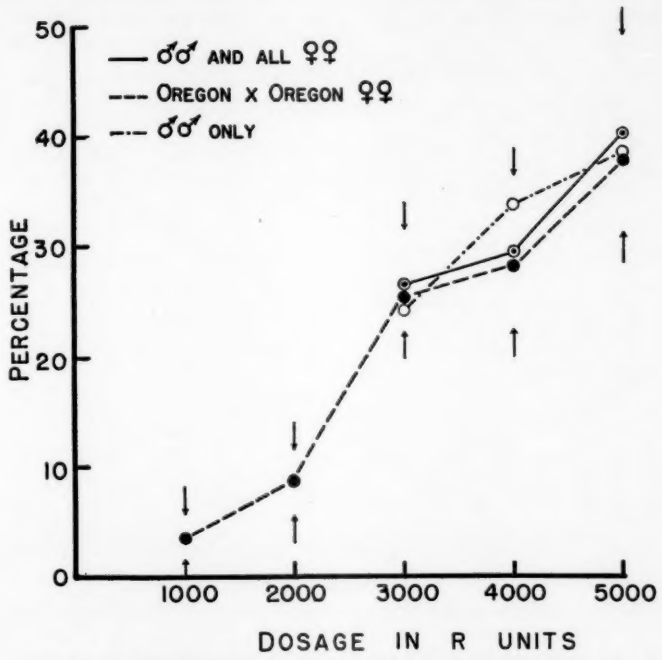

FIGURE 1.—Relation between dosage and the percentage of altered sperm. Arrow tips indicate double values of standard errors.

these data is given in table 1. In this summary is included material from all experiments, namely from females and males with both parents Oregon-R, as well as from females and males of which the female parent was Swedish-b and the male parent Oregon-R. These three sets of data are separated in the graph shown in figure 1. In this figure the relation between the dosage and the percentage of altered sperm is shown graphically. The arrow points on this chart indicate the values of double the standard errors. The curve resembles an S-shaped curve, being steepest in the interval between 2000 and 3000 r-units. Although the standard errors are still high, the observed values show a significant deviation from a linear propor-

tionality obtained in experiments where the frequency of lethals was used as a criterion. Since this is the first occasion in an extensive X-ray experiment that a deviation from linear proportionality is observed, it might be well to wait for confirmation of these results before passing a final judgment as to the significance of this finding.[1] It is evident from the chart shown in figure 1 that the 3000 r-units point is the only one significantly upsetting the straight line arrangement. It might well be that some uncontrolled experimental factor is responsible for the unusual position of that point.

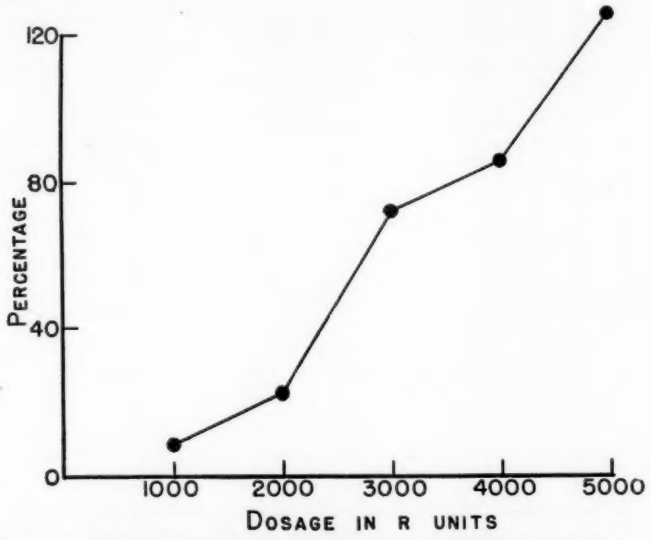

FIGURE 2.—Relation between dosage and the percentage of breaks per total sperms.

The relation between the dosage and the total number of breaks observed is shown graphically in figure 2. The curve rises steeply between 2000 and 3000 r-units and there seems to be no decrease at the highest dosages, although larger numbers would be required to prove this point. These data also show no linear relationship between the dosage and the effect.

The data summarized in table 1 show that with the increase in dosage the proportion of changed sperm increases rapidly. These data indicate also a similar relationship for the average number of breaks per changed sperm. For example, among the changed sperm treated with 1000 r-units, 2.25 ± 0.13 breaks per sperm were observed while among such sperm

[1] Note added in proof: In a paper just published, Sax (1938) has found a similar disproportionality between dosage and observed breaks in irradiated chromosomes of Tradescantia.

treated with 5000 r-units, 3.13 ± 0.16 breaks per sperm were found. The data show a trend of increase in the frequency of multiple breaks with increase in dosage, although their absence at low dosages may possibly be due to the low number of total breaks observed in these groups.

Distribution of breaks among chromosomes.—The frequency with which the single chromosomes and single chromosome limbs take part in the breakage processes is summarized in table 2. The frequency of X and Y

TABLE 2

Break percentage and chromosome length.

	BREAKS IN X SPERMS		BREAKS IN Y SPERMS		TOTAL BREAKS IN AUTOSOMES		BREAKS IN X SPERMS	SALIVARY GLAND NO. BANDS (BRIDGES)	SALIVARY GLAND LENGTH		MITOTIC GONIAL (GOWEN & GAY)	METAPHASE NEUROCYTES (COMBINED DATA)
	n	%	n	%	n	%	%	%	%		%	%
2L	142	18.3±1.4	43	16.4±2.3	185	17.7	38.7	37.3	17.8	37.9	32.2	36.5
2R	158	20.4±1.5	77	29.4±2.8	235	22.5			20.1			
3L	161	20.7±1.5	35	13.4±2.1	196	18.8	39.5	40.9	18.3	42.0	40.9	38.8
3R	146	18.8±1.4	58	22.1±2.6	204	19.5			23.7			
4	11	1.4±0.4	5	1.9±0.8	16	1.5	1.4	1.3	1.4	1.4	4.1	2.0
X	158	20.4±1.5					20.4	20.5	18.7	18.7	22.8	22.7
Y			44	16.8±2.3								

breaks can be determined in only one class of larvae, namely X breaks in females and Y breaks in males. The breaks in autosomes are obtained from both types of larvae. Since autosomes represent approximately 80 percent of the total length of all chromosomes, the percentages of breaks for autosomes was calculated on that basis. The five long strands of the female larvae show approximately the same frequency of breaks, which also seems characteristic for the Y chromosome (KAUFMANN AND DEMEREC 1937). A comparison between the number of breaks and the number of bands in salivary chromosomes, length in salivary chromosomes, and the length in mitotic metaphases indicates a general agreement for all comparisons, although the correspondence is better in the case of salivary chromosomes.

Distribution of breaks along chromosomes.—The distribution of breaks along the chromosomes is given in tables 3 and 4. The positions of breaks have been determined within the limits of the divisions indicated on BRIDGES' (1935) maps. In table 3 data are arranged according to divisions, and in table 4 according to the number of breaks found in them. Cases in

TABLE 3

Distribution of breaks along the chromosomes (sfa stands for spindle-fiber attachment region).

DIVISION	n	DIVISION	n	DIVISION	n	DIVISION	n
1	10	21	11	61	19		
2	7	22	11	62	12		
3	7	23	9	63	7		
4	5	24	5	64	16		
5	8	25	9	65	11		
6	6	26	12	66	11		
7	9	27	6	67	17		
8	8	28	6	68	7		
9	8	29	10	69	7		
10	4	30	9	70	6		
11	12	31	6	71	9		
12	13	32	8	72	6		
13	5	33	10	73	4		
14	11	34	8	74	2		
15	3	35	13	75	13		
16	2	36	10	76	6		
17	1	37	3	77	7		
18	5	38	3	78	3		
19	9	39	7	79	6		
20	25	40	29	80	27		
sfa		sfa		sfa		sfa	
		41	48	81	23	101	9
		42	20	82	7	102	7
		43	7	83	6		
		44	12	84	6		
		45	7	85	12		
		46	8	86	12		
		47	10	87	11		
		48	5	88	10		
		49	10	89	9		
		50	11	90	10		
		51	9	91	5		
		52	8	92	9		
		53	11	93	8		
		54	4	94	17		
		55	10	95	5		
		56	14	96	16		
		57	7	97	8		
		58	2	98	12		
		59	16	99	6		
		60	16	100	12		

which it was impossible to determine the position of a break with reasonable accuracy have been omitted from the list.

There have been found from 1 to 48 breaks per division. The distribution of break frequencies (table 4) approximates a typical variability curve except for divisions adjacent to the spindle-fiber attachment. These di-

visions are partially or totally heterochromatic, and all except the 101 region of the fourth chromosome show high break frequencies. The values for the euchromatic divisions indicate in general a uniform distribution of breaks. The value of P for the X chromosome calculated by the χ^2 method is 0.05.

In evaluating this and the following tables, it should be kept in mind that the division by BRIDGES is not based on actual size units. The same

TABLE 4

Distribution of breaks along the chromosomes. (Heterochromatic divisions are in italics.)

FEMALES ONLY:

NUMBER OF BREAKS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 ... 19	19 ... 22	22 ... 24	25 ... 33
DIVISIONS	17	16	15	10	4	6	2	5	7	1	14	11	12	61	94	*81*	*80*	*40*	*20*	*41*
	58	38	24	28	13	30	3	8	19	29	35	59		64						
	74	48	31	32	18	46	23	9	33	62	42	96		67						
			37	52	27	63	25	21	44		60	100								
			45	54	30	79	36	22	49		75									
			70	57	47	86	43	26	65											
			73	76	51	89	68	34	66											
			78	82	72	92	69	50												
				83	77	93	85	53												
				91	84	97	102	55												
				95	88	*101*		56												
				99	98			71												
								87												
								90												
	3	3	8	12	12	11	10	14	7	3	5	4	1	3	1	1	1	1	1	1

FEMALES AND MALES:

NUMBER OF BREAKS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17 ... 19	20 ... 23	23 ... 27	27 ... 29	29 ... 48
DIVISIONS		58	37	54	24	27	30	32	23	29	21	26	35	56		59	67	61	42	*81*	*80*	*40*	*41*
		74	38	73	48	28	43	34	25	33	22	44	75			60	94						
			78		91	31	45	46	30	36	50	62				64							
					95	70	57	52	51	47	53	85				96							
						72	63	93	71	49	65	86											
						76	68	97	89	55	66	98											
						79	69		92	88	87	100											
						83	77		*101*	90													
						84	82																
						99	102																
		2	3	2	4	10	10	6	8	8	7	7	2	1		4	2	1	1	1	1	1	1

number of breaks in two different sections, therefore, may be caused either by actual equality of size, or in case of inequality by preferred breakage in the shorter section. It is difficult to correlate the data on breaks with the size of the different sections because the maps of BRIDGES are not completely true to the actual size relations. Some chromosome regions have been relatively overstretched (for instance the middle part of 3R). Nevertheless, there is some indication of preferred breakage of some regions. As seen in table 4, the terminal sections of all chromosomes fall in break

TABLE 5

Break frequency in distal sections.

	DIVISIONS	NO. BREAKS	PERCENTAGES OF TOTAL EUCHROMATIC LIMB			RATIO BREAKS:LENGTH
			BREAKS	BANDS	LENGTH	
X	1–2	17	12.78	9.98	9.44	1.35:1
2L	21–22	22	14.10	8.77	9.28	1.52:1
2R	59–60	32	17.11	11.16	10.23	1.67:1
3L	61–62	31	18.34	11.84	11.35	1.61:1
3R	99–100	18	9.94	7.20	7.06	1.40:1

classes above the average value. This exceptional behavior is still more emphasized by checking the cytological length of the sections all of which are shorter than the average. Table 5 gives the values of break percentages and length relations for all terminal regions (two terminal sections in each case) as compared with the respective euchromatic chromosome limbs (sections 1–19 and the comparable ones in the autosome limbs). The ratio between break number and length which should be 1 in case of completely even distribution of breaks, however, is in all cases around 1.5. Though none of the values is statistically significant, the general trend in all chromosomes is clear and speaks in favor of preferred occurrence of breaks in the most distal chromosome parts.

The other exception is the high break number of the proximal sections except in chromosome 4. The cause of this higher break frequency may be (1) an inherent property of these regions to break more easily than euchromatic parts, or (2) a different coiling state of the chromonema as compared with that in euchromatic parts. The first possibility is based on the assumption that the salivary gland chromosomes represent completely uncoiled chromonemata. The comparison between the break numbers and the relative length of heterochromatic parts in mitotic metaphase (table 6) shows a good correspondence between break number and heterochromatin

TABLE 6

Comparison of frequency of breakage and mitotic length in heterochromatic regions of X and autosomes.

	PERCENTAGE OF BREAKS	ESTIMATED PERCENTAGE OF LENGTH OF CHROMOSOME	
		METAPHASE	SALIVARY
X	15.8	33.3	5
2	18.3	16.6	5
3	12.5	12.5	5

TABLE 7

Distribution of multiple breaks among the chromosome limbs.

NO. BREAKS	DISTRIBUTION	NO. OF CASES			PER CENT			P
					OBSERVED		EXPECTED	
		♀♀	♂♂	TOTAL	♀♀	♀♀ & ♂♂		
2	1, 1	97	47	144	61.78	65.16	80	less than
	2	60	17	77	38.22	34.84	20	0.01
3	1, 1, 1	2	6	8	7.41	19.51	48	less than
	2, 1	20	8	28	74.07	68.30	48	0.01
	3	5	—	5	18.52	12.19	4	
4	2, 1, 1	19	5	24	47.50	48.00	57.6	
	1, 1, 1, 1	6	4	10	15.00	20.00	19.2	0.14
	3, 1	6	—	6	15.00	12.00	12.8	
	2, 2	9	1	10	22.50	20.00	9.6	
	4	—	—	—	—	—	0.8	
5	2, 1, 1, 1	2	1	3				
	2, 2, 1	4	2	6				
	3, 2	6	—	6				
6	2, 2, 1, 1	5	1	6				
	3, 1, 1, 1	—	1	1				
	4, 1, 1	1	—	1				
7	3, 2, 1, 1	1	1	2				
	4, 1, 1, 1	—	1	1				
	3, 2, 2	1	—	1				
8	6, 2	1	—	1				
Unanalyzable		26	—	26				
Alterations involving the 4th chromosome		15	3	18				

length for the two autosomes, while the break number in the X heterochromatin is considerably lower. Of course, it must be realized that the determination of heterochromatic breaks may be incomplete, because, as stated above, not all intraheterochromatic rearrangements are recognizable by our method.

Distribution of multiple breaks.—Regarding the distribution of multiple breaks among different chromosomes, another exceptional behavior is revealed. Leaving aside chromosome 4, chance distribution of two breaks among five chromosomes with equal average break frequency (the limbs of the long autosomes have been dealt with as separate chromosomes) would

result in 20 percent of inversions (both breaks in the same chromosome) and 80 percent of translocations (both breaks in different chromosomes). In case of 3 breaks similar calculations give 48 percent of cases with 3 breaks in 3 different chromosomes, 48 percent of cases with 2 breaks in one and 1 break in another chromosome, and only 4 percent with all 3 breaks in one chromosome. These data can be derived from the formula

TABLE 8

Distance of break points in inversions and translocations (2 break cases only).

REARRANGEMENTS IN EUCHROMATIC REGIONS	BREAK DISTANCE IN DIVISIONS																			NO. OF CASES	P
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Inversions Observed	3	5	6	6	4	7	4	1	4	4	—	—	1	—	2	—	1	—	1	49	0.61
		20				16				8				3			2				
Expected		*16.5*				*14.9*				*10.3*				*5.7*			*1.6*				
Translocations Observed	7	8	8	10	7	3	4	3	6	5	4	7	5	5	1	1	3	1	—	88	0.25
		33				17				22				12			4				
Expected		*29.7*				*26.8*				*18.5*				*10.2*			*2.8*				
Totals Observed		53				33				30				15			6			137	0.48
Expected		*46.2*				*41.7*				*28.8*				*15.9*			*4.4*				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Totals Observed	10	13	14	16	11	10	8	4	10	9	4	7	6	5	3	1	4	1	1	137	0.70
		64				41					25						7				
Expected		*57.7*				*46.9*					*25.2*						*7.1*				

$(a+b+c+d+e)^n$, a to e representing the five chromosomes in percent of the length (or break probability) of the chromosome set, *n* indicating the number of breaks. In our case the small autosome may be disregarded for sake of simplification and the five long strands may be taken as equal in length and breakability. The actual values vary from 17.7 to 22.5 (table 2). These differences from the assumed equality in break frequency are too small (giving about 0.1 percent deviation from the ratio 80:20 in 2-break cases) to affect the calculations. In table 7 all data are summarized according to the distribution of the breaks among the chromosomes. The first vertical column classifies breaks according to numbers observed in single nuclei, the second column subdivides these classes according to their distribution among chromosome limbs, namely 1, 1 in the 2-breaks class signifies that each of the two breaks observed in these nuclei occurred in a different limb of the chromosome set, while 2 signifies that both breaks occurred in the same limb. Other columns of that table show frequencies of various classes.

In the cases with 2, 3, and 4 breaks the data indicate that the observed distribution does not agree with expectation. In all these cases the least probable combinations occur with high frequencies. The same holds true for cases with higher break numbers for which data are not sufficient to be expressed in percentages. For instance, the only fully analyzable case with 8 breaks has an expectation frequency of less than 0.1 percent. That the observed frequencies differ significantly from the expected distribution is shown in the last columns which give the probabilities with which such distributions would be expected to occur by chance.

TABLE 9

Distribution of types of rearrangements.

NUMBER OF BREAKS	COMBINATION OF BREAKS	DOSE IN r UNITS					TOTAL	
		1000	2000	3000	4000	5000	OBSERVED	EXPECTED
4	2+2	—	3	17	11	9	40	9.5
	4	—	—	6	1	3	10	40.5
5	2+3	—	1	6	1	5	13	
	5	—	—	1	—	1	2	
6	2+2+2	—	—	2	—	1	3	
	3+3	—	—	1	—	—	1	
	2+4	—	—	2	1	1	4	
	6	—	—	—	—	—	—	
Higher numbers and unclear cases		1	1	9	5	10	26	

Distance between breakage points.—In table 8 data are arranged to show the length of inversions and the distance between breakage points in translocations expressed as the difference of length from the breakage point to the spindle fiber attachment. In this summary, two-break cases only have been included, as it is possible that higher breaks within one sperm would obscure the results. Cases in which one break occurred within the heterochromatic region were omitted since the possibility is not excluded that the true relationship between lengths in heterochromatin and euchromatin might be different than expressed in salivary chromosomes. The top line in table 8 indicates classes expressed in divisions of BRIDGES' (1935) map of distance between two breaks. In the other columns are given the observed frequencies for each class and for groups of four classes together with the expected frequencies for four class groups. At the lower end of the table, classes are summarized in groups of five in order to increase the number of cases in each group. Probabilities (P) calculated by the χ^2 method show a rather good correspondence between observed and expected values, indicating that two breaks in euchromatic regions occur at random, namely independently of each other.

Distribution of types of rearrangements.—If random combination takes place between all break ends, some of the rearrangements will simulate simultaneously occurring independent rearrangements. For instance, four breaks will give a certain number of cases with two independent inversions or translocations among the majority which represents complicated exchanges involving all eight break ends. The ratio between both these types (2+2 and 4) is different with different distribution of the breaks among the chromosomes, due to differences in the number of viable recombinations. If the breaks have occurred in four different limbs (1, 1, 1, 1), the ratio 2+2:4 will be 3:6. In 2, 1, 1-cases it will be 3:14, and in the other combinations (2, 2; 3, 1; and 4) 3:22. A test of our data (table 9) shows that the actual relation between single complicated and multiple independent rearrangements is just the reverse. In all break classes the cases with several independent rearrangements, each with 2 or 3 breaks only, overweigh by far. No clear 6-break case has been found representing a simultaneous rearrangement among all break ends. In table 9 the expected values are calculated for 4-break distributions only, since the data in other cases are not large enough to warrant such calculations.

Rearrangements and contact points.—In order to consider the mechanism through which various observed rearrangements may be accomplished,

TABLE 10

Analysis of the rearrangements in terms of the "contact hypothesis."

DOSAGE IN r UNITS	NUMBER OF "CONTACT POINTS" REQUIRED						
	2	3	4	5	6	7	8
1000	8	3	—	—	—	—	—
2000	23	6	—	—	—	—	—
3000	171	27	8	1	—	—	1
4000	67	9	2	—	—	—	—
5000	77	19	6	1	1	—	—
Totals	346	64	16	2	1	—	1
	80.5%	19.5%					

it is helpful to determine the number of strands which should be in contact at one point, provided such a condition is essential that recombinations between strands in contact may occur. In table 10 data are summarized to show the number of contact points. The first line of that table gives classes indicating the number of strands required to be in contact at one point in order that certain observed rearrangements may be accomplished. In the other part of the table observed frequencies for different classes are listed. It is evident that in 19.5 percent of the cases more than two strands would

be required to be in contact and one case has been analyzed in which a contact of eight strands would be required to furnish the observed rearrangement.

DISCUSSION

The possible mechanisms of the origin of induced chromosomal rearrangements have been discussed by several authors, notably SEREBROVSKY (1929), and STADLER (1932).

Chromosomal rearrangements depend on separation of preexisting genic connections and reunion in new combinations. A priori two alternative explanations are possible. (1) Free breakages are independently induced and reunion in twos follows later (STADLER). In this event different consequences are to be expected dependent on whether the process of reunion is limited in space and time or not. If it is, then the results will not differ much from those expected on the basis of the second possibility, which is that (2) breakage and reunion are part of one process and occur simultaneously. Here again two rather divergent mechanisms can be, and have been, proposed. In the first place, (2a) external attachment may precede both breakage and reunion which then will take place in mechanical ways only at regions of original contact (SEREBROVSKY). On the other hand, (2b) broken ends independently induced as in (1) may, by nature of their "unsaturated" attraction forces, cause the rupture of intact chromosomes with which they lie in contact. While the mechanism (2a) would work at microscopic dimensions, the latter could be effective at molecular distances only, that is in places where the chromosomes are as closely apposed as the homologues are in meiotic synapsis.

X-ray treatment would act in (1) and (2b) by increasing the breakage number and thus augmenting the number of rearrangements. In (2a) it would lead to a change of the general, perhaps colloidal, state of the nucleus by which the chromosomes become "sticky." For this possibility there is a remarkable parallel in the case of homozygous sticky plants in *Zea mays*. BEADLE (1937) has shown that in the progeny of these, the normal mutation rate is raised approximately ten times as compared with heterozygous, or wild type plants. This increase is observable in both chromosomal rearrangements as well as in apparent gene mutations. The cause in this case is clear from direct observations. The chromosome substance is changed by the sticky gene in such a way as to become gluey, thus giving all kinds of attachments. That similar changes can be the result of X-ray treatment has recently been shown by WHITE (1937).

The first point in considering the different possibilities cited above, is to determine whether random combination between breakage ends occurs or not. The distribution of the breakages seems to be random among and

along the chromosomes with two possible exceptions. (1) The behavior of heterochromatic parts is still open to discussion. KAUFMANN and DEMEREC (1937) from their data on break frequencies in Y sperms, have reached the conclusion that there is in all chromosomes including the Y a breakage frequency directly proportional to their metaphase length. From this they further conclude that the chromonema length is directly proportional to metaphase length, as suggested by MULLER and PAINTER (1932); moreover that the Y therefore is of the same general structure as euchromatic chromosomes, and that direct proportionality exists between chromonema length and breakage numbers in all chromosome parts. They are forced to consider the relative length of hetero- and euchromatic regions in salivary gland chromosomes to be secondarily disturbed by differential uncoiling, the heterochromatic parts and the Y chromosome remaining in a state of submicroscopic folding. Cytological tests are not yet available. They might be obtained, however, from observations in polarized light. The opposite hypothesis put forward by MULLER and GERSHENSON (1935) assumed that the salivary gland chromosomes represent the real proportions of chromonema length of the different sections, and that in mitosis the relative length of eu- and heterochromatin is changed due to non-spiralization of the heterochromatin. This hypothesis seems to have found support by the genetic evidence obtained by MULLER and coworkers (MULLER, RAFFEL, GERSHENSON and PROKOFYEVA-BELGOVSKAYA 1937; MULLER, PROKOFYEVA-BELGOVSKAYA and RAFFEL 1938) on the differential effect of duplications and corresponding deficiencies on certain parts of the inert region of the X chromosome in its metaphase length. The so-called block "A," though a small part of the heterochromatin only, seems to be mainly responsible for the length of the inert region in mitosis, while on the basis of KAUFMANN and DEMEREC'S assumption metaphase length should be proportionally affected by these duplications.

Our data pertaining to this problem are summarized in table 6, from which it is evident that, for autosomes, there is a close agreement between observed values for breaks and the length of heterochromatic regions as measured in metaphase chromosomes. However, the break value for the X chromosome is intermediate between that expected from metaphase length and that expected from salivary length. It has been pointed out that inversions and translocations with both breaks in the heterochromatic region cannot be detected by the method used in this study. If metaphase length is assumed to represent equivalent chromonema lengths in all regions, then the length of the heterochromatin of X should be equivalent to about nine euchromatin divisions. The data shown in table 8 indicate that about 90 percent of inversions occur within a distance of nine divisions and from the data for 2-break cases shown in table 7, it may be inferred

that about 35 percent of breaks are connected with such inversions. By taking into account all these factors, an adjustment for undetectable inversions can be made which brings the figure up from 15.8 to about 22.0 percent. The adjusted figure is still appreciably below the expected 33.3 percent, although an additional correction for undetectable heterochromatic translocations would bring it still closer to the expected value. The significance of this discrepancy may possibly be determined through additional data from experiments which are now in progress. It seems advisable to refrain from passing judgment on this question until the additional evidence becomes available and for the present to consider both possibilities, namely that (1) salivary chromosomes give a true picture of the actual length of the heterochromatic region and (2) that the true picture is evident from metaphase chromosomes.

The other possible exception to random distribution of breaks is the break frequency of distal ends, which is slightly, but consistently, higher than that of the other euchromatic sections.

The interpretation of these results must be different according to the different possible mechanisms. If breaks are produced independently, then we would observe only a fraction of them, the realized breaks, while others must have disappeared by fusion of the original breakage ends. The observed differences from random distribution, therefore, may be due to both higher frequency of induced breaks and lower probability of disappeared breaks.

The second point concerning the arrangement of chromosomes in the sperm head is whether random exchange occurs between all chromosome parts. Several facts are revealed from our data. First, the different types of break combinations show differences from expectancy. Accumulation of breaks within one chromosome is evident for 2-break cases and highly probable in case of more breaks (table 7). Secondly, as far as analyzable (table 9), four and more breaks very rarely lead to a simultaneous exchange among all altered chromosomes, but are dissolvable into two or more independent rearrangements each involving fewer breakage points. This result contradicts the data collected by DUBININ and KHVOSTOVA (1935), but as they have dealt with a selected group of aberrations their results do not seriously affect our conclusion.

These facts taken together indicate that the combination of break loci is not at random but is restricted. No attempt will be made to define more precisely what spatial arrangement is responsible for the different phenomena. But the general cause of them must be that rearrangements are limited in space and time.

No sign of a very constant arrangement of the chromosomes is obvious. PATTERSON, STONE, BEDICHEK and SUCHE (1934) have obtained results

from which they arrived at a very special picture of the architecture of the sperm-head. All ends, proximal and distal ones, are assumed to be polarized towards the same part of the nucleus. This picture was derived from the fact that translocation breakage occurred preferably in the spindle fiber regions and in the distal ends. Our data are not in accord with theirs. The reason may partly be that a number of translocations with both breaks in the chromocentral parts escaped us due to the chosen method. The apparent increase of breaks in the distal ends in PATTERSON'S material, much higher than that we have found, may partly be a consequence of the differences between the cytological and the genetical maps.

From the results on the location of breakage points it is not possible to decide between the different schemes for the origin of rearrangements. Our data indicate, however, that breakage and recombination must be local phenomena.

KIRSSANOW (1937), from a study of the percentage relation between reciprocal and triple translocations, believes he has obtained decisive evidence against the break-hypothesis and for the so-called crossing-over hypothesis, our alternative 2. In his calculations, however, he reckons only with the possibility of free combination in case of induced free break ends. In light of the results presented in the present paper, his positive decision in favor of the contact hypothesis is unwarranted.

From the types of chromosomal rearrangements, conclusions have been drawn as to the mechanism of their origin. One of the most obvious points is that single breakage rearrangements have been found only very rarely, none in the present study. Even if the distal ends of chromosomes are for internal reasons incapable of accepting free fragments, terminal deficiencies should occur more often. Their absence in most cases has led several authors, especially MULLER and his associates to doubt the very existence of 1-breakage cases, that is, terminal deficiencies. MULLER believes that, the mechanism of origin of rearrangements being somehow related to the crossing-over process, two breaks is the minimum number inducible. This conclusion, however, has not been proved. Even if it were assumed that there are no terminal deficiencies among the stocks, and that all described cases, especially those of DEMEREC and HOOVER (1936), may be regarded as representing 2-breakage cases with the distal break very near the tip, the possibility remains that the absence of such deficiencies may be due to elimination rather than to non-occurrence. This elimination of course is not due in all cases to hypoploidy but may be a consequence of the behavior of broken ends of chromosomes, such as CARLSON (1938) has described in irradiated neuroblast cells of grasshoppers. If new free ends are unstable and tend to reattach themselves to other similar free ends, then the two chromatids of the same chromosome with the original terminal deficiency will unite and thus form a bridge at next anaphase. This bridge

will break again, and there is no reason to believe the new connection between both ends will be weaker than other places, so that the next break will take place by chance leading to two broken chromosomes of different lengths. This process must recur at many or all following divisions, thus gradually increasing the differences in genic content of the daughter cells. Thus, inviable combinations will necessarily result and in the end the 1-breakage cases will be eliminated. Therefore, their absence does not disprove possibility (1) of their origin. On the other hand, the multiple cases are rather difficult to explain on the basis of possibility (2b). At any given point a close contact between two chromosomes may reasonably be assumed but not between more. As indicated in table 10, rearrangements requiring more than two contact points constitute about 20 percent of the total. One case has been analyzed requiring contact of eight strands. On the other side, DUBININ and KHVOSTOVA (1935) have offered another variation of the contact hypothesis by suggesting that multiple rearrangements take place in parts of the nucleus where the presumptive breakage regions have become entangled into a knot. As in this case knot-formation is independent of the following irradiation one would expect that the same relative amount of multiple breakage-cases would occur with all dosages. Our data, although not extensive enough to warrant a general conclusion, suggest, however, that multiple breaks occur more frequently at higher dosages.

Important for further considerations is the relation between X-ray dosage and percentage of aberrations. For the different possibilities of the origin of rearrangements, different curves might be expected. A linear relation would result in (2b) if the induced breaks are a direct consequence of the ionization. An exponential curve will result from a mechanism suggested by STADLER at least for those dosages which do not induce more than two breaks in one sperm. Higher breakage numbers and limited combination possibilities will lead to more complicated relations. According to possibility (2a) probably an S-shaped curve as typical for chemical reactions might be expected.

Table 1 and figures 1 and 2 suggest that a direct proportionality does not exist between dosage and the number of sperms with changes, the number of aberrations, and the number of individual breakages. Suggestive is the strong increase between 2000 and 3000 r units. The trend of the resulting curve is not determinable owing to the large standard errors.

These results contradict certain published data. HEPTNER and DEMIDOVA (1936) believe that from 1000 to 4000 r units there exists a direct linear relation for point mutations, deficiencies and other rearrangements as well. The separation between the different types and their grouping, however, seems not clear thus making their data unreliable. Of a different nature is the direct proportionality found by KHVOSTOVA and GAVRILOVA

(1935) for translocations showing position effect for *ci*+. This result is significant but does not necessarily mean that the translocations result from a mechanism elaborated under (2b) but can well be interpreted by the hypothesis of STADLER (1). In these special cases two breakages of different probabilities are involved. One breakage necessarily must be next to *ci*+, the other, however, may be anywhere along the euchromatic limbs of the other chromosomes or in the heterochromatin of X or in the Y. The probability for this second type of breakage, therefore, is much higher, so that practically in every case when a break will have occurred near *ci* there is a second one available for recombination. Nearly every breakage at *ci* then will lead to a rearrangement. As in these cases a direct proportionality was observed this suggests that either the rearrangement as a whole or the independent break is directly induced. The other results presented here do not favor the first explanation and, therefore, justify the following conclusions, that (1) breakages are independently induced by the ionization in a way comparable to point mutations, as assumed by STADLER, and that (2) recombination between free breakage ends occurs in a limited space and time.

One possible objection to these conclusions is the fact that small deficiencies involving one band are rather frequent (SLIZYNSKA 1938). They would have to be interpreted as representing two breaks occurring very close together, but their actual number seems to be much higher than that predictable on the basis of chance occurrence of such breakages. For this reason the other possibility must be held open, namely that short deficiencies have an origin not related to that of other chromosomal aberrations but more similar to that of point mutations. The frequent occurrence of induced chromosomal aberrations with lethal effects might then be due to one point of the rearrangement representing a true break, the other one being an independent deficiency, as has been suggested by DEMEREC (1937).

Data obtained by DEMEREC (1938) indicate that Oregon-R stock, the same one which was used in these experiments, has a low induced mutation rate when compared with Swedish-b stock and with the other material for which data are published. In the experiments referred to, 1000 r-units induced 1.30 ± 0.24 sex linked lethals, 3000 gave 2.15 ± 0.45, 4000 produced 3.08 ± 0.47, and 5000 r-units induced 5.43 ± 0.76 lethals. When these genetic results, showing the relation between dosage and the frequency of induced X chromosome lethals, are compared with the cytological results given in table 1 and figure 1, showing the relationship between dosage and the frequency of sperms with chromosomal aberrations, it can be seen that cytological values for 3000 to 5000 dosages are slightly higher than genetic values, while the value for 1000 r-units is appreciably lower. The figures of table 1 represent aberrations observed in all chromosomes. In order to

obtain values for the X chromosome only, these figures should be reduced to 18.7 percent. Recalculated figures are 0.68, 1.62, 4.98, 5.57, 7.50 percent for 1000, 2000,3000, 4000, and 5000 r-units respectively. It is known that not all chromosomal aberrations are connected with lethals and that not all lethals are connected with chromosomal aberrations. Evidence is also available that the incidence of aberrations is disproportionally higher among lethals induced by stronger treatment (DEMEREC 1937). It is of interest to note that a similar relationship between aberrations and lethals is evident here. At 1000 r-units the value for lethals is higher than the value for aberrations, while at higher dosages the reverse is true. This relationship could be explained by assuming that a similar mechanism is responsible for both lethals and aberrations, namely that a portion of the lethals originate at a breakage point. If chromosomal rearrangements are formed through breakages and subsequent reattachments, then at low dosages, where breaks are rare and occur mostly singly in individual nuclei, a broken chromosome would be restored by a reattachment, while at higher dosages, which induce multiple breaks in nuclei, such reattachments may produce chromosomal rearrangements. Thus it is to be expected that low dosages would give a higher proportion of lethals not connected with chromosomal aberrations and therefore that the relationship between values for lethals and values for aberrations would be different at low and at high dosages.

The data shown in table 8 indicate that distances between two breaks are distributed at random, namely that two breaks occurring in the same chromosome are independent of each other. Since it is definitely known that in case of crossing over the position of the second break is influenced by the position of the first break, the present results suggest that a different mechanism is responsible for reattachments connected with crossing over than for those producing induced inversions and translocations.

SUMMARY

Chromosomal rearrangements were studied by salivary gland chromosome analysis of 1765 F_1 larvae obtained from untreated females mated to X-rayed males. A total of 1038 breaks were plotted in euchromatic regions within the limits of the single divisions of BRIDGES' (1935) maps.

The data indicate a significant deviation from the straight line proportionality between dosage and frequency of breaks.

Distribution of breaks among chromosomes of comparable lengths is at random.

In general, the distribution of breaks is at random within euchromatic sections of chromosomes, with the possible exception of distal regions where breaks tend to be slightly more frequent. In heterochromatic regions, breaks are more frequent then in euchromatic regions for similar salivary chromosome lengths.

Two breaks occurring in euchromatic regions are independent of each other. In case of multiple breaks a tendency is noticeable for them not to be distributed at random among chromosomes but to accumulate within chromosomes.

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ON THE INHERITANCE AND EXPRESSION OF A MOTTLED-EYED MUTANT IN DROSOPHILA MELANOGASTER[1]

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Received July 28, 1938

INTRODUCTION

MOTTLED-EYED flies have been reported on various occasions by numerous investigators. The following paper presents further data on the inheritance and expression of a mottled-eyed mutant of *Drosophila melanogaster*. The mutant first appeared in a progeny of flies which had been subjected to supersonic vibrations in an experiment conducted by HERSH, KARRER and LOOMIS (1930), in an attempt to induce mutation. In this experiment there appeared five mottled-eyed males in the progeny of 26,135 flies, resulting from matings of treated cut forked Bar males with virgin double-yellow females with attached X chromosomes. This fact suggested sex linkage. The mottled males were bred to the double-yellow sisters and the flies in the F_1 were normal in each case. Upon allowing the F_1 to interbreed some mottled flies of both sexes appeared in the F_2. The character was then carried along with a greater or smaller number occurring in each generation.

The mutant was later crossed with a vermilion stock by DR. W. P. SPENCER thus producing a stock of vermilion mottled-eyed flies. Such was the nature of the stock at the beginning of this investigation.

The mutant shows a pronounced dark mottling of the eyes and was illustrated by SURRARRER (1935). These illustrations show variations from almost complete dark pigmentation to nearly entire removal. At temperatures of 25°C and above complete removal of the dark pigmentation results. Not only is there variation in the magnitude of the effect, but also a considerable variation in the compactness of the darkened area.

Because of this variability and frequent lack of the character expression under ordinary culture conditions, it was necessary to find control methods for production. The author found (1935) that the character was expressed at temperatures of 20°C or below. It was further found that the temperature-effective period was in the pupa stage and that the percentage of the area of the eye affected was inversely proportional to the developmental temperature.

If the main problem of genetics and development is the determination

[1] Submitted to the Division of Biology of Western Reserve University in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Sincere appreciation is here expressed to Dr. A. H. Hersh of Western Reserve University under whose direction the work was accomplished.

of the intermediate action between the genes at the beginning and the external characters at the end of development, such a mutant presents rather unique material for a study of genic expression. With a controlled procedure for the expression of the character there was furnished the necessary information for further study of the genetics of the mutant.

PROCEDURE AND RESULTS OF GENETICAL ANALYSIS

The flies were cultured in large stock bottles on 1 percent agar agar banana media. They were placed at 27°C for increased egg production and rapid larval development. Before the beginning of pupation the adults were removed and the cultures placed at 18°C for further development. Mottling may be prevented from developing in the adults by subjecting their pupae to temperatures of 25°C or above. The question immediately arises, will such red individuals, thus produced, have the same hereditary qualities as the mottled adults, if the pupae are subjected to the same environmental conditions.

Mass cultures of the mottled mutant were allowed to develop at 27°C. Before the time of pupation the adults were removed, and part of the cultures placed at 18°C for further development. Upon emergence two mass cultures were made up from the phenotypically non-mottled adults which had been produced at 27°C, and two like cultures from the above mentioned phenotypically mottled adults produced at 18°C. The offspring thus produced, in accord with general expectation, showed no appreciable difference in character expression.

The localization of the mottled gene[2] in its respective chromosome was carried out with a physiologically balanced stock *S/Cy D*/C III X. Mottled virgin flies were crossed to *S/Cy D*/C III X males. The F_1 without exception was non-mottled. This confirmed the recessive nature of the mutant and further proved the character was not sex linked. The Star Dichaete F_1 males were crossed back to mottled females, and there appeared in the progeny Star mottled, but no Dichaete mottled flies. This showed that mottled is in the third chromosome. This fact is further confirmed by crossover data.

Selected Dichaete males from the above described cross were mated to virgin mottled flies. The following results were obtained from the first backcross.

D/mot ♂♂ × *mot/mot* ♀♀

Dichaete	mottled	Normal
123	119	10

The appearance of normal in the offspring was assumed to be due to modifiers. This is partly proved by the results of the next back-cross.

[2] This gene has been given the symbol *mot*[28] but for the sake of simplicity is referred to throughout this paper as *mot*.

D/mot ♂♂ × *mot/mot* ♀♀

Dichaete	mottled	Normal
280	251	0

Assuming mottled to be a typical Mendelian recessive, the above data, even though small, fall within acceptable deviation.

	Dichaete	mottled
Observed	280	251
Expectation	265.5	265.5
Difference	14.5	14.5

From the formula $\sigma = \sqrt{qnp}$ it is found that the probable error is within acceptable limits.

In the isolation of the Dichaete flies for the above crosses it was noticed that Star flies were usually only slightly mottled. The influence of Star was checked by producing mottled Star flies and then backcrossing to mottled. The offspring from such a cross were carefully checked. In order to illustrate the comparative degree of mottling the following method of estimation was used. When $\frac{3}{4}$ of the area of the eye was estimated to be pigmented, it was recorded as 4, when approximately $\frac{1}{2}$ as 3, less than $\frac{1}{2}$ as 2, and when only slightly pigmented as 1.

	S/+mot/mot ♀♀ × *+/+mot/mot* ♂♂							
	S/+mot/mot				*+/+mot/mot*			
Area mottled	1	2	3	4	1	2	3	4
Individuals	42	11	3	0	1	6	29	41

From the above data, it seems that Star acts as an inhibitor to the area of the eye effected by mottled.

After the homozygosity was such that the offspring from *mot/mot* ♀♀ × *D/mot* ♂♂ were either mottled or Dichaete, reciprocal crosses were made to determine the crossover value between mottled and Dichaete. Mottled males were crossed to *D/mot* females, resulting in offspring as illustrated in table 1.

TABLE 1

Crossover data involving Dichaete and mottled
D/mot ♀♀ × *mot/mot* ♂♂.

DICHAETE	MOTTLED	NON-DICHAETE NON-MOTTLED	DICHAETE MOTTLED
802	666	50	35 (Total 1555)

From the data it is evident that Dichaete flies are apparently more viable than mottled ones. It was expected that the crossover columns

III and IV would more closely approach each other; yet it would be logical to assume that the phenotypically normal flies would be more viable than the mutants. According to the foregoing data the crossover value of mottled and Dichaete is 5.46 percent. From this result mottled is either in close proximity to the spindle fiber or is in the approximate position of rose.

The cross *D/mot*×*mot/mot* used in the attempt to determine the position of mottled from Dichaete was allowed to inter-breed during the summer of 1936 from June until August. At the end of the three months' period virgin mottled crossed to Dichaete males gave as in the previous work either Dichaete or mottled offspring in accord with expectation. Dichaete virgin flies from the above lines were chosen and crossed to the mottled males from the same strain, *D/mot* ♀ ♀ ×*mot/mot* ♂ ♂ resulting in some mottled Dichaete flies. From such crosses the mottled Dichaete male and female flies were carefully selected, and each sex placed in shell vials and crossed back to the mottled line. In this way a mottled Dichaete strain was easily maintained by crossing Dichaete mottled males to mottled females with constant selection of the Dichaete mottled males.

A Stubble line of *D. melanogaster* was obtained from Pasadena in 1935 with the third chromosome constitution *S*/C3 13a. These flies were crossed to mottled flies which had been inbred with the Dichaete line previously mentioned. (+/+ +/+ *Sb*/C3 13a +/+)×(+/+ +/+ *mot/mot* +/+)→+/+ +/+ *Sb/mot* +/+ and +/+ +/+ *mot*/C3 13a +/+. The Stubble males were carefully selected and crossed back to the previously mentioned mottled flies. The F_1 generation showed a predominance of Stubble and thus to increase the homozygosity of the lines the Stubble males were chosen and crossed back to the mottled females of the previously mentioned line for seven generations. In all cases Stubble flies outnumbered the expectation of a 1:1 ratio and in no case did the non-Stubble exceed the fifth backcross in which the non-Stubble flies comprised 45.7 percent of the progeny.

The Stubble males of the seventh backcross were carefully selected and crossed to virgin Dichaete mottled females.

Sb/mot ♂ ♂ ×*D mot/mot* ♀ ♀

Ten such crosses were made up on 1 percent agar banana media in large culture bottles and incubated as previously described for character expression. The *D mot/*++*Sb* females thus produced were crossed to *mot/mot* males. Ten of these crosses were made up with approximately 10 females and 20 males in each bottle. At the end of three days the adults were removed and placed in new culture bottles for an additional three day egg laying period, and incubated as above.

The flies from the previously mentioned crosses were carefully checked

under a Spencer binocular with a 23× objective and 9× ocular combination. The results of the cross are listed in table 2.

TABLE 2

Crossover data involving Dichaete, mottled and Stubble.

$$\frac{D\ mot\ +}{+\ +\ Sb} \ \text{♀♀} \times \frac{+\ mot\ +}{+\ mot\ +} \ \text{♂♂}$$

NON-CROSSOVERS				CROSSOVERS											
D mot + / *+ + Sb*				*D*(1) *mot*(2) *+* / *+ + Sb*											
1		1		2		2		1:2		1:2					
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
343	418	1339	1589	81	66	39	54	22	27	109	120	3	4	100	128

Total 4442

From table 2 it is evident that all of the expected flies occurred in the population. It is further evident that in all cases except the Dichaete Stubble flies the viability of the females is slightly above that of the males. This is, however, probably not significant, and somewhat in accord with the expectation.

The distorted ratio of the non-crossover flies (761 Dichaete mottled to 2929 Stubble) is probably significant. The expected ratio is 1:1 and the result is 1:3.85. Because of the low viability of the Dichaete mottled flies when in competition with Stubble, the Dichaete mottled crossover percent has not been considered of value.

The crossover value of Stubble and mottled as determined from table 2 is as follows:

D / *+*	*mot* / *mot*	*Sb* / *+*	Dichaete *mot Sb*	49
+ / *+*	*+* / *mot*	*+* / *+*	Normal	229
D / *+*	*+* / *mot*	*+* / *+*	Dichaete	228
+ / *+*	*mot* / *mot*	*Sb* / *+*	mottled Stubble	7
				513

$$513 \div 4442 = 11.54\%$$

To further confirm the position of mottled by the crossover method the *Sb/mot* males were again selected and crossed back to mottled females to further increase homozygosity. The viability of Stubble was again, as in all previous cases, above that of mottled.

Mottled males were then crossed back to Stubble heterozygous mottled females.

$$Sb/mot\ ♀♀ \times mot/mot\ ♂♂$$

Seven such crosses were made up on 1 percent agar banana media in large stock bottles. Ten females with about twenty males were placed in each bottle and incubated as in previous experiments.

TABLE 3

Crossover data involving mottled and Stubble
Sb/mot ♀♀ × mot/mot ♂♂.

	NON-CROSSOVERS				CROSSOVERS			
	MOTTLED		STUBBLE		MOTTLED STUBBLE		NORMAL	
	♂	♀	♂	♀	♂	♀	♂	♀
Totals	178	195	546	580	26	30	61	93

From the data in table 3 there is a total of 1709 flies with 210 crossovers between *Sb* and *mot*.

$$210 \div 1709 = 12.2\%\ \text{crossover value}$$

This crossover value compares with the previous mottled, Stubble crossover value within 1.66 units.

The crossover value as determined when Stubble heterozygous mottled and Dichaete mottled were mated was as follows:

D + *Sb* / + *mot* +	Dichaete Stubble	147
+ *mot* + / + *mot* +	mottled	93
D + + / + *mot* +	Dichaete	228
+ *mot* *Sb* / + *mot* +	mottled Stubble	7
		475

$$475 \div 4442 = 10.6\%$$

Thus mottled is placed between 47.66 and 51. However, as previously stated, the crossover value of Dichaete and mottled when obtained in competition with Stubble is probably not valid. The Stubble mottled crossover value, on the other hand is apparently valid. Thus the mottled Stubble crossover value when determined independently of Dichaete is 12.2; while that obtained in combination with Dichaete is 11.54. The combined data give a value of 11.87.

Even though all the crossover data do not exactly conform, it is evident that mottled is very close to the spindle fibre of chromosome three. The spindle attachment has been placed on the standard map at 46. From the above, the data indicate mottled to be between 45.86 and 46.33; the mean of which is 46.08.

Thus from the foregoining data the mutant is definitely placed on chromosome three as shown by a dominant physiologically balanced stock and further confirmed by the above linkage data. The three point linkage data place the character in close proximity to the spindle fiber attachment. It was found by GLASS (1933) in X-ray induced dominant mosaic eye-color mutants that chromosome breaks were at or very near the locus involved and further that in chromosome II and III frequent breaks occur in the vicinity of the spindle fiber. SCHULTZ (1936) believes that a translocation of inert regions from the spindle attachment or other loci to active regions is the probable reason for variegation.

Whether the character involves a visible translocation or inversion will only be definitely determined by cytological salivary analysis.

THE TEMPERATURE EFFECTIVE PERIOD AND ACTUAL EXPRESSION

The author (1935) showed that the temperature effective period of the mottled mutant was during the first half of the time interval between pupation and emergence. A further analysis of the development was undertaken in an attempt to more accurately place the temperature effective period of the character.

PROCEDURE AND RESULTS

Large families of flies were made up on 1 percent agar agar banana media in straight $2\frac{3}{4} \times 3\frac{3}{4}$ inch Stender glasses. Such containers were used to facilitate recovery of pupae. These cultures were maintained at 27°C. The adults were removed upon the appearance of the larvae and the containers carefully watched until pupae development approached a peak. All existing cases were then removed and from that time pupae were taken out at hourly intervals.

This experiment was carried out in an underground room where temperature fluctuation was rather slight. It was thus possible to maintain a

temperature under a goose neck lamp of approximately 27°C during the removal of the pupae. The pupae were carefully taken out by the use of a wire loop and placed on a narrow strip of paper toweling which extended down into approximately 3 cc. of water in the bottom of the test tube. The tubes were then plugged with cotton in the usual manner. In this way a rather constant humidity was maintained which seemed to satisfy conditions for future emergence. The tubes were numbered and immediately returned to the 27°C incubator. Each tube then remained at 27°C for a definite time interval ranging from 24 to 39 hours, preliminary experiments having revealed the effective period to be within these limits. The data recorded in table 4 were obtained from two sets of nine families each. The data from each set overlapped the other as to time interval. From the two sets of data a total of 662 flies of known age is recorded. It becomes evident that the temperature effective period falls between 25 and 35 hours of development under these controlled conditions.

TABLE 4

Data on the temperature effective period of mottled and non-mottled as observed by hourly transfers from 27°C. to 18°C.

Hours at 27°C	24		25		26		27		28		29	
	mot	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*
Individuals	8		20		50	3	31	1	32	17	10	26
Hours at 27°C	30		31		32		33		34			
	mot	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*		
Individuals	6	37	4	31		51	1	60	2	71		
Hours at 27°C	35		36		37		38		39			
	mot	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*	*mot*	*non-mot*		
Individuals	75		43		29		32		31			

The visible expression of the mutant is, however, much later. This time was determined in the following way. Pupae were embedded in modeling clay on the white stage of a binocular microscope. In this way the anterior portion of the pupa case was carefully removed with a pair of very sharp needles, thus exposing the developing eye. It was soon found in dissecting pupae cases of different ages that the deposition of the brown pigmentation preceded the vermilion. This is exemplified in the photo-

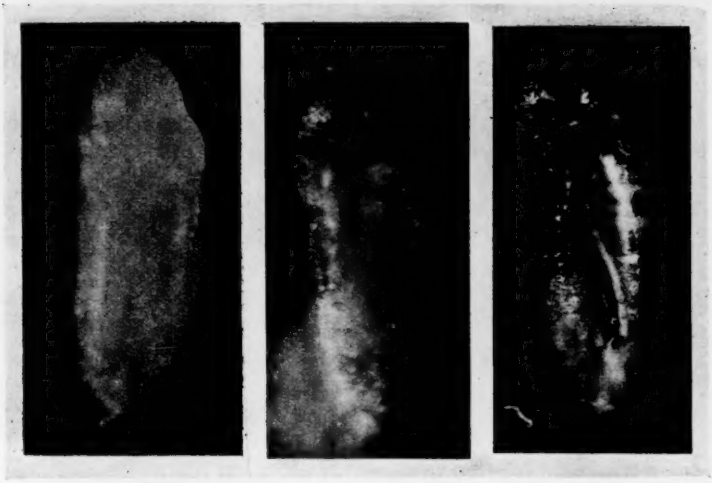

FIGURE 1.—Photomicrographs of dissected pupae showing (A) no pigmentation, (B) dark brown deposition and (C) dark brown deposition and vermilion pigmentation.

micrographs (fig. 1) of such dissected pupae. These photomicrographs were taken through a 4 mm. Bausch and Lomb microscope objective directly

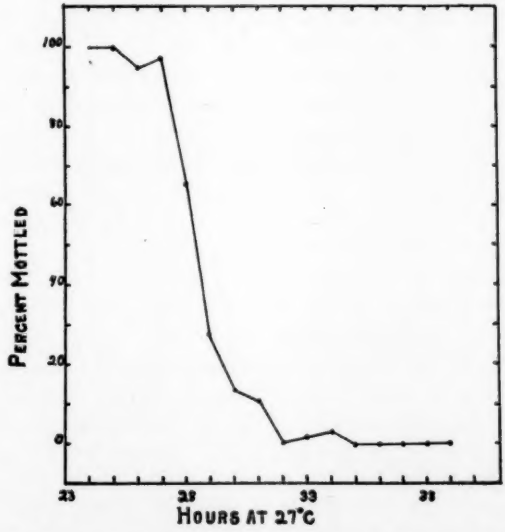

FIGURE 2.—Graphic relation of the temperature effective period.

adapted in place of the lens on a $3\frac{1}{4}\times4\frac{1}{4}$ inch plate camera. A shows no eye coloration, B shows the dark pigmentation but no vermilion, and C shows the dark brown pigmentation and the incoming vermilion coloration.

Figure 2 is a graphic representation of the percentage of flies examined which showed the mutation at various hours of exposure to a temperature of 27°C.

It was observed that the dark brown pigmentation on the eye did not spread in extent after it once became visible, but gradually became more sharply differentiated from surrounding tissue.

Large families of flies were again made up, as previously described, for spontaneous pupae production. The cases were removed at hourly intervals and handled as in the previously described fashion. After the pupae were incubated at 27°C for 24 hours they were placed at 18°C. At the end of an additional 48 hours the pupae were carefully embedded in modeling clay, which had previously been molded into suitable shape on a removable Spencer binocular stage plate. The clay and plate were placed in the incubator so that their temperature would be that of the pupae. As the pupae were successfully dissected they were placed on microscope slides in definite positions, which had previously been determined by two rows of numbers. In this way the exact age of the pupae could be easily recorded. When the slide was filled with pupa cases it was slipped on to a carrier which was made of two strips of glass with an air space of about $\frac{1}{2}$ inch between them. Both the carrier and the numbered slides had been incubated at 18°C so that the pupae would not change temperature greatly when mounted. This was an attempt to have a means of observation at future hourly intervals without significant temperature changes. The slides were slipped off the carrier after each observation and both slides and carrier remained in the incubator until the next observation.

The first group of pupae were taken at six different consecutive hourly intervals. They were dissected and observed at hourly intervals for color deposition at room temperatures (22°–25°C). The second group of pupae were taken at eight different consecutive hourly intervals. They were dissected and observed at hourly intervals for color deposition in a cold room (10°–15°C, during December).

It became evident in these two experiments that the hourly observations were more frequent than necessary, because hourly changes were not recordable and further it changed environmental conditions unnecessarily. The third group of pupae were all taken at a single hourly interval. They were dissected and observed at three hour intervals in a constant 18°C thermostatically controlled chamber. The data of the three experiments are recorded in table 5.

From these data it becomes evident that temperature is of vital importance in the time of pigment deposition of both brown and vermilion. In the first group of 12 pupae taken at six different consecutive hourly

TABLE 5

Recorded data of the time of brown and vermilion deposition in individuals of experiments I, II and III.

TIME OF INDIVIDUAL BROWN COLORATION AFTER PUPATION (8 HR. INTERVALS)			TIME OF VERMILION COLOR APPEARANCE (3 HR. INTERVALS)		
I	II	III	I	II	III
87	96	91	102	114	109
87	99	94	105	114	109
89	100	94	104	127	109
90	103	94	110	127	109
90	103	94	108	127	109
90	103	94	105	127	109
91	103	94	103	130	109
91	104	94	100	116	109
91	104	94	106	122	109
92	104	94	110	122	109
92	104	94	110	125	109
101	104	94	107	125	109
	104	94		125	112
	104	94		125	112
	104	94		125	112
	104	94		125	112
	104	94		128	112
	105	94		120	112
	105	94		126	112
	106	94		115	112
	106	97		127	109
	106			130	
	108			123	
	108			126	
	108			126	
	108			129	
	108			129	
	112			127	
	112			127	
Mean *90.8*	*104.4*	*94*	*105.8*	*124.1*	*110.9*

intervals there is a range from 87 to 101 hours in the time of the deposition of brown, or a difference of 14 hours. The vermilion color change varies from 100 to 110 hours after pupation, a difference of 10 hours. The dissection of pupae and interval of observation under a binocular were carried out at 22°–25°C. Thus the cases were at a temperature above 18°C

during some of their development and the rate of change would be expected to be increased. The second group of 29 individuals show a range of from 96 to 112 hours in the time of the brown deposition or a difference of 16 hours. The vermilion color change varies from 114 to 130 hours—a range of 16 hours. The dissection of pupae and of observation were carried out in a cold room 10° to 15°C. Thus the temperature of the pupae was below

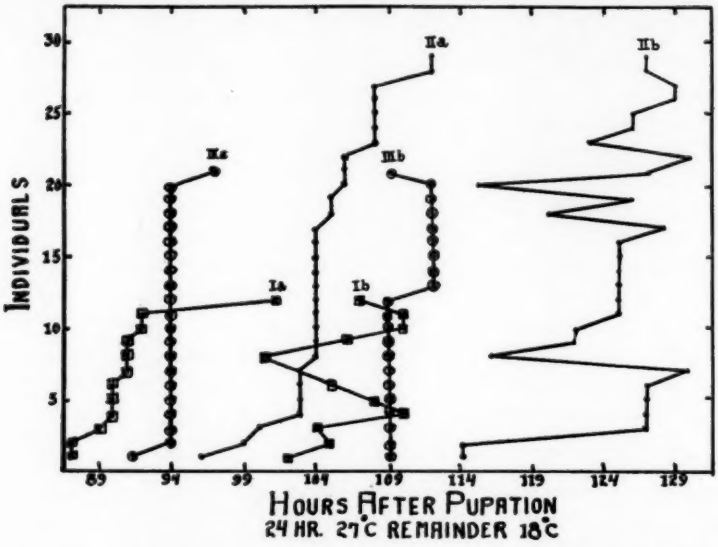

FIGURE 3.—Graphic relation of experiments I, II, and III. Ia, IIa, and IIIa indicate the time at which the various individuals showed brown deposition. Ib, IIb, and IIIb indicate the time the same individuals showed vermilion deposition.

18°C during some of their development and the rate of change would be expected to be decreased.

If the temperatures of observation and dissection were of significance, the third group of pupae dissected and observed at 18°C should fall between the other two. Such is the case as is shown graphically in figure 3. The third group of 21 pupae were all taken at the same hour interval and show a range of 6 hours for brown and 3 for vermilion.

There are, however, other reasons for the variation. In groups I and II the pupae were taken at six and eight different consecutive hourly intervals respectively. Thus on no two occasions would environmental conditions be identical. The degree of injury of an individual at the time of dissection and the time at which it occurred would undoubtedly retard or accelerate certain processes. Nevertheless, it becomes evident that the actual ap-

pearance of the mottling occurs long after the temperature effective period for its development. This is indicated in figure 4, in which the mean of the effective period obtained from 662 individuals falls at 30 hours (±5 hrs.). The mean of actual appearance of brown and vermilion obtained from 21 individuals of the third group is indicated by the horizontal line (24 hours at 27°C and the remainder of development at 18°C). Thus the mean point

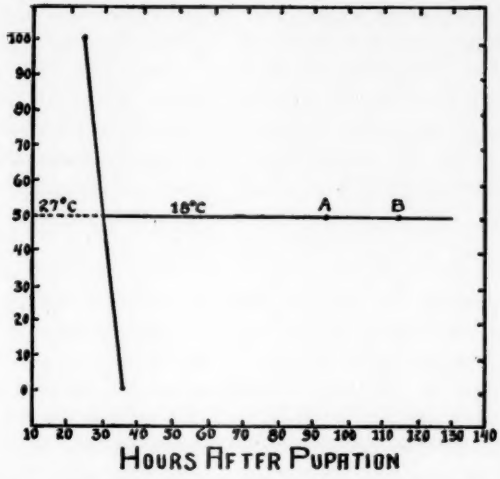

FIGURE 4.—The horizontal line is the graphic relation (A) of the mean time of actual brown deposition; (B) the mean time of the actual vermilion deposition to the temperature effective period.

of brown deposition falls at 94 hours, and that of vermilion at 110.9 hours after pupation. (It should be remembered that observations are recorded at three hour intervals, and the range of brown deposition is from 91–97 hours and vermilion from 109–112.)

DISCUSSION

The mottled mutant here described is one of extreme environmental sensitivity. It was shown by the author (1935) that at temperatures of 20°C or below the brown pigmentation was expressed; while at temperatures of 25°C or above it was erased.

The character is sharp in its expression. Neighboring facets are either darkly pigmented or free from the pigment entirely. This undoubtedly means that no dilution occurs. Apparently the molecular size of the pigment is sufficient to be unable to diffuse to neighboring facets. The number of facets effected and thus the size of the area of brown pigmentation dep-

osition is roughly inversely proportional to the temperature (SURRARRER 1935). The difference in the color of the eye is in accord with changes in temperature apparently in the manner of a chemical reaction. Comparable changes as a result of temperature have frequently been recorded with special reference to wing and eye mutants in Drosophila.

The temperature effective period falls within the pupae stage and is between 25 and 35 hours after pupation at 27°C. This period is the time limit from the first individual to the last to show the temperature effect.

The actual appearance of the brown pigmentation as would be generally expected falls definitely after the sensitive period. Under the most carefully controlled conditions it appears between 91 and 97 hours; while the vermilion deposition comes considerably later, between 109 and 112 hours. (It should be kept in mind that a three hour observation interval was used.) These data conform with the results of SCHULTZ (1935) which showed that in eye mutants of dark pigmentation the color deposition was definitely ahead of the light or more dilute eye mutants.

If genic action as expressed by mutants is the acceleration or retardation of normal processes, the mottled vermilion combination affords fortunate experimental material. SCHULTZ (1935) showed in Drosophila that the sequence of pigment deposition in the eye was yellow to tan to red. This is also confirmed by the work of COCHRANE (1936) in *Drosophila pseudo-obscura*, which shows further that eye color may be the effect produced by two or more genes, each acting in its own sensitive period. Thus the early appearance of the brown deposition (the temperature effective period of which is known) in reference to vermilion (whose temperature effective period is not known) would physically obstruct the appearance of the latter.

Mottling of the eyes in Drosophila may be attributed to several types of changes or various combinations of them.

(1) Point mutation or chromosome change, although always a possibility apparently has no substantial proof. MULLER (1930) states that visible variations induced by X-rays which involve point mutations are similar to spontaneous visible variations; and that such simple point mutations are stable in their inheritance. No eversporting cases have been found.

(2) Somatic disjunction or reduction of chromatin provided possible explanation for MORGAN (1911) and SPENCER (1926, 1930).

(3) Somatic crossing over and segregation (STERN 1935).

(4) Inversion or translocation resulting in probable somatic elimination of chromatin (PATTERSON 1928, 1932, 1933; PATTERSON and PAINTER 1931; VAN ATTA 1932; GOWEN and GAY 1934).

(5) Inversion or translocation resulting in a positional change of inert

material from the region of the spindle fiber to other active loci; or perhaps better, the relations of inert material to active loci (GOWEN and GAY 1933; SCHULTZ 1936).

(6) Unstable gene hypothesis (DEMEREC and SLIZYNSKA 1937).

A frequently recorded type is that of X-ray induced translocation with apparent somatic elimination. It seems probable that the mottled here concerned is a somewhat stable nuclear change, highly dependent upon environmental conditions. This seems evident because (1) at 20°C or below the character is expressed in 100 percent of a population; while at temperatures of 25°C or above it is completely absent. (2) The lower the temperature the greater is the area of the eye affected. It seems that the molecular change in the formation of the pigment is only possible at relatively low temperatures. (3) The time of the temperature effective period is sufficiently late in the cycle to remove the possibility of somatic change. (4) Flies whose eyes are non-mottled as a result of the temperature effect, produce mottled offspring if the pupae are held at temperatures of 20°C or below.

Mottled is on the third chromosome and may involve more than a point mutation. It is evident, however, that the region of the spindle fiber is involved. The temperature effective period of the mutant is rather sharply defined as is also the time of its actual expression.

SUMMARY

1. Mottled acts as a typical Mendelian recessive and is very sensitive to temperature.

2. Adults with the mottled character erased because of development at 25°C or above produce progeny with mottling expressed if the pupae develop at suitable temperatures.

3. Mottled is on the third chromosome as located by the dominant physiologically balanced stock *S/Cy D*/C IIIX. This is further confirmed by linkage data.

4. By three point linkage data the mottled character was placed at 5.46 crossover units from Dichaete and 11.87 units from Stubble. It thus involves the region of the spindle fiber attachment.

5. Mottled is a rather stable nuclear change, and not a result of somatic elimination or mutation.

6. The temperature effective period falls between 25 and 35 hours after pupation at 27°C.

7. The expression of mottled occurs 91 to 97 hours after pupation (24 hours at 27°C and the remaining development at 18°C). The vermilion coloration occurs between 109 and 112 hours after pupation (24 hours at 27°C and the remaining development at 18°C).

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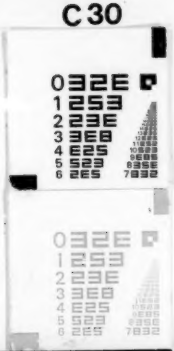
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